Supplemental Information for manuscript

Title: Large-scale interlaboratory study to develop, analytically validate and apply highly multiplexed, quantitative peptide assays to measure cancer-relevant proteins in plasma

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Table of Contents for Supplemental Information

Click on the green link with the page numbers to be directed to the corresponding page.

Supplemental Table S1A: 27 heavy (U-^{15}N) proteins and light (unlabeled) proteins used as internal standards, plus 7 additional light proteins used as digestion control

Protein #	SwissProt	Protein Description	species	protein U15N (heavy)	protein light
1	P09972	Fructose-bisphosphate aldolase C	human	yes	yes
2	P04083	Annexin A1	human	yes	yes
3	P09525	Annexin A4	human	yes	yes
4	P20073	Annexin A7	human	yes	yes
5	Q53G71	Calreticulin variant (Fragment)	human	yes	yes
6	O00299	Chloride intracellular channel protein 1	human	yes	yes
7	P15311	Ezrin	human	yes	yes
8	Q16658	Fascin	human	yes	yes
9	P02792	Ferritin light chain	human	yes	yes
10	P39748	Flap endonuclease 1	human	yes	yes
11	P09211	Glutathione S-transferase P	human	yes	yes
12	Q04760	Lactoylglutathione lyase	human	yes	yes
13	P62993	Growth factor receptor-bound protein 2	human	yes	yes
14	P04792	Heat shock protein beta-1	human	yes	yes
15	Q14116	Interleukin-18	human	yes	yes
16	P09382	Galectin-1	human	yes	yes
17	O00151	PDZ and LIM domain protein 1	human	yes	yes
18	P32119	Peroxiredoxin-2	human	yes	yes
19	Q13162	Peroxiredoxin-4	human	yes	yes
20	P23297	Protein S100-A1	human	yes	yes
21	P29034	Protein S100-A2	human	yes	yes
22	P04271	Protein S100-B	human	yes	yes
23	P54727	UV excision repair protein RAD23 homB	human	yes	yes
24	O76070	Gamma-synuclein	human	yes	yes
25	P09493	Tropomyosin alpha-1 chain	human	yes	yes
26	O00762	Ubiquitin-conjugating enzyme E2 C	human	yes	yes
27	P63279	SUMO-conjugating enzyme UBC9	human	yes	yes
28	P00974	Pancreatic trypsin inhibitor	bovine	no	yes
29	P41160	Leptin	mouse	no	yes
30	P68082	Myoglobin	horse	no	yes
31	P02687	Myelin basic protein	bovine	no	yes
32	P07288	Prostate-specific antigen	human	no	yes
33	P00433	Peroxidase C1A	horseradish	no	yes
34	P02741	C-reactive protein	hman	no	yes

Supplemental Table S1B: Peptides derived from heavy and light proteins used as internal standards, and corresponding 13C/15N synthetic peptides

Protein Name	Peptide Sequence	Precursor Charge	**light** Precursor Mz	**heavy** Precursor Mz	**heavy label**	**all N15** Precursor Mz
sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	600.83	605.83	R10	609.30
sp|P09972|ALDOC_HUMAN	AEVNGLAAQGK	2	529.29	533.29	K8	536.26
sp|P09972|ALDOC_HUMAN	ELSDIALR	2	458.76	463.76	R10	464.24
sp|P09972|ALDOC_HUMAN	TPSALAILENANVLAR	2	826.97	831.97	R10	837.44
sp|P09972|ALDOC_HUMAN	QVLFSADDR	2	525.76	530.77	R10	532.25
sp|P04083|ANXA1_HUMAN	ALYEAGER	2	454.73	459.73	R10	460.21
sp|P04083|ANXA1_HUMAN	GTDVNVFNTILTTR	2	775.91	780.92	R10	785.38
sp|P04083|ANXA1_HUMAN	GVDEATIIDILTK	2	694.39	698.39	K8	701.37
sp|P04083|ANXA1_HUMAN	AAYLQETGKPLDETLK	3	592.98	595.66	K8	599.30
sp|P04083|ANXA1_HUMAN	TPAQFDADELR	2	631.80	636.81	R10	639.28
sp|P09525|ANXA4_HUMAN	VLVSLSAGGR	2	479.79	484.79	R10	486.27
sp|P09525|ANXA4_HUMAN	GLGTDEDAIISVLAYR	2	846.94	851.95	R10	856.42
sp|P09525|ANXA4_HUMAN	DEGNYLDDALVR	2	690.33	695.33	R10	698.30
sp|P09525|ANXA4_HUMAN	GLGTDDNTLIR	2	587.81	592.81	R10	595.28
sp|P09525|ANXA4_HUMAN	GAGTDEGCLIEILASR	2	831.41	836.42	R10	840.88
sp|P20073|ANXA7_HUMAN	LYQAGEGR	2	447.23	452.23	R10	453.21
sp|P20073|ANXA7_HUMAN	SEIDLVQIK	2	522.80	526.81	K8	528.28
sp|P20073|ANXA7_HUMAN	GAGTDDSTLVR	2	546.27	551.27	R10	553.25
sp|P20073|ANXA7_HUMAN	EFSGYVESGLK	2	608.30	612.31	K8	614.28
sp|P20073|ANXA7_HUMAN	GFGTDEQAIVDVVANR	2	845.92	850.93	R10	856.39
sp|Q53G71|Q53G71_HUMAN	QIDNPDYK	2	496.74	500.74	K8	502.22
sp|Q53G71|Q53G71_HUMAN	GLQTSQDAR	2	488.25	493.25	R10	495.23
sp|Q53G71|Q53G71_HUMAN	FYALSASFEPFSNK	2	804.39	808.40	K8	812.37
sp|Q53G71|Q53G71_HUMAN	EQFLDGDGWTSR	2	705.82	710.82	R10	714.29
sp|Q53G71|Q53G71_HUMAN	GQTLVVQFTVK	2	610.36	614.36	K8	617.34
sp|O00299|CLIC1_HUMAN	LHIVQVVCK	2	548.32	552.33	K8	554.80
sp|O00299|CLIC1_HUMAN	YLSNAYAR	2	479.24	484.25	R10	485.23
sp|O00299|CLIC1_HUMAN	IEEFLEAVLCPPR	2	786.91	791.91	R10	794.88
sp|O00299|CLIC1_HUMAN	GVTFNVTTVDTK	2	641.34	645.34	K8	648.32
sp|O00299|CLIC1_HUMAN	GFTIPEAFR	2	519.27	524.28	R10	525.26
sp|P15311|EZRI_HUMAN	IQVWHAEHR	2	588.31	593.31	R10	597.28
sp|P15311|EZRI_HUMAN	EDEVEEWQHR	2	678.79	683.80	R10	687.27
sp|P15311|EZRI_HUMAN	SGYLSSER	2	449.72	454.72	R10	455.20
sp|P15311|EZRI_HUMAN	IALLEEAR	2	457.77	462.77	R10	463.25
sp|P15311|EZRI_HUMAN	SQEQLAAELAEYTAK	2	826.41	830.42	K8	835.39
sp|Q16658|FSCN1_HUMAN	YLTAEAFGFK	2	573.80	577.80	K8	579.28
sp|Q16658|FSCN1_HUMAN	FLIVAHDDGR	2	571.80	576.81	R10	579.28
sp|Q16658|FSCN1_HUMAN	YLAPSGPSGTLK	2	595.82	599.83	K8	602.31
sp|Q16658|FSCN1_HUMAN	VTGTLDANR	2	473.75	478.76	R10	480.23
sp|Q16658|FSCN1_HUMAN	LSCFAQTVSPAEK	2	719.36	723.36	K8	726.83
sp|P02792|FRIL_HUMAN	LGGPEAGLGEYLFER	2	804.41	807.42	R6	813.38
sp|P02792|FRIL_HUMAN	KPAEDEWGK	2	530.26	533.27	K6	536.24
sp|P39748|FEN1_HUMAN	HLTASEAK	2	428.73	432.74	K8	434.21
sp|P39748|FEN1_HUMAN	WSEPNEEELIK	2	687.33	691.34	K8	694.31
sp|P39748|FEN1_HUMAN	SIEEIVR	2	423.24	428.24	R10	428.23
sp|P39748|FEN1_HUMAN	QLQQAQAAGAEQEVEK	2	864.43	868.44	K8	875.40
sp|P39748|FEN1_HUMAN	LIADVAPSAIR	2	563.33	568.34	R10	570.31
sp|P09211|GSTP1_HUMAN	FQDGDLTLYQSNTILR	2	942.48	947.48	R10	953.45
sp|P09211|GSTP1_HUMAN	YISLIYTNYEAGK	2	767.89	771.90	K8	775.37
sp|P09211|GSTP1_HUMAN	EEVVTVETWQEGSLK	2	867.43	871.44	K8	876.41
sp|P09211|GSTP1_HUMAN	PPYTVVYFPVR	2	669.37	674.37	R10	676.35
sp|P09211|GSTP1_HUMAN	ASCLYGQLPK	2	568.79	572.80	K8	574.77
sp|Q04760|LGUL_HUMAN	FSLYFLAYEDK	2	698.35	702.35	K8	704.33
sp|Q04760|LGUL_HUMAN	SLDFYTR	2	451.22	456.23	R10	456.21

sp|Q04760|LGUL_HUMAN	IAWALSR	2	408.74	413.74	R10	414.22
sp|Q04760|LGUL_HUMAN	GFGHIGIAVPDVYSACK	3	597.63	600.31	K8	604.28
sp|Q04760|LGUL_HUMAN	FEELGVK	2	411.22	415.23	K8	415.21
sp|P62993|GRB2_HUMAN	FNSLNELVDYHR	3	502.92	506.25	R10	509.23
sp|P62993|GRB2_HUMAN	FGNDVQHFK	2	546.27	550.27	K8	553.25
sp|P62993|GRB2_HUMAN	NYVTPVNR	2	481.76	486.76	R10	488.24
sp|P62993|GRB2_HUMAN	ATADDELSFK	2	548.76	552.77	K8	554.25
sp|P62993|GRB2_HUMAN	ESESAPGDFSLSVK	2	726.85	730.85	K8	734.32
sp|P04792|HSPB1_HUMAN	LFDQAFGLPR	2	582.31	587.32	R10	589.29
sp|P04792|HSPB1_HUMAN	AQLGGPEAAK	2	471.26	475.26	K8	477.24
sp|P04792|HSPB1_HUMAN	VSLDVNHFAPDELTVK	3	595.31	597.98	K8	601.96
sp|P04792|HSPB1_HUMAN	DGVVEITGK	2	459.25	463.26	K8	464.24
sp|P04792|HSPB1_HUMAN	QLSSGVSEIR	2	538.29	543.29	R10	545.27
sp|Q14116|IL18_HUMAN	EDELGDR	2	417.19	422.19	R10	422.17
sp|Q14116|IL18_HUMAN	SDIIFFQR	2	513.27	518.28	R10	519.26
sp|Q14116|IL18_HUMAN	TIFIISMYK	2	558.31	562.32	K8	563.30
sp|Q14116|IL18_HUMAN	ISTLSCENK	2	526.26	530.27	K8	531.74
sp|Q14116|IL18_HUMAN	GMAVTISVK	2	453.26	457.27	K8	458.25
sp|P09382|LEG1_HUMAN	DGGAWGTEQR	2	538.74	543.75	R10	546.22
sp|P09382|LEG1_HUMAN	DSNNLCLHFNPR	3	496.23	499.57	R10	502.88
sp|P09382|LEG1_HUMAN	FNAHGDANTIVCNSK	3	549.92	552.59	K8	556.90
sp|P09382|LEG1_HUMAN	SFVLNLGK	2	439.26	443.27	K8	444.25
sp|P09382|LEG1_HUMAN	LPDGYEFK	2	484.74	488.75	K8	489.23
sp|O00151|PDLI1_HUMAN	CGTGIVGVFVK	2	568.81	572.82	K8	574.79
sp|O00151|PDLI1_HUMAN	GCTDNLTLTVAR	2	660.83	665.84	R10	668.81
sp|O00151|PDLI1_HUMAN	VAASIGNAQK	2	479.77	483.78	K8	486.25
sp|O00151|PDLI1_HUMAN	VWSPLVTEEGK	2	622.83	626.84	K8	629.31
sp|O00151|PDLI1_HUMAN	DFEQPLAISR	2	588.31	593.31	R10	595.29
sp|P32119|PRDX2_HUMAN	GLFIIDGK	2	431.76	435.76	K8	436.24
sp|P32119|PRDX2_HUMAN	TDEGIAYR	2	462.72	467.73	R10	468.21
sp|P32119|PRDX2_HUMAN	LSEDYGVLK	2	512.27	516.28	K8	517.26
sp|P32119|PRDX2_HUMAN	ATAVVDGAFK	2	489.77	493.77	K8	495.25
sp|Q13162|PRDX4_HUMAN	DYGVYLEDSGHTLR	3	542.26	545.59	R10	548.57
sp|Q13162|PRDX4_HUMAN	LVQAFQYTDK	2	606.82	610.82	K8	613.30
sp|Q13162|PRDX4_HUMAN	IPLLSDLTHQISK	3	488.95	491.62	K8	494.60
sp|Q13162|PRDX4_HUMAN	QITLNDLPVGR	2	613.35	618.35	R10	621.32
sp|P23297|S10A1_HUMAN	ELLQTELSGFLDAQK	2	846.45	849.46	L6	855.42
sp|P29034|S10A2_HUMAN	ELPSFVGEK	2	503.27	506.28	K6	508.25
sp|P29034|S10A2_HUMAN	YSCQEGDK	2	493.70	496.71	K6	498.68
sp|P04271|S100B_HUMAN	AMVALIDVFHQYSGR	2	853.94	856.95	R6	864.41
sp|P04271|S100B_HUMAN	ELINNELSHFLEEIK	3	609.99	611.99	K6	616.63
sp|P54727|RD23B_HUMAN	EQVIAALR	2	450.27	455.27	R10	456.25
sp|P54727|RD23B_HUMAN	IDIDPEETVK	2	579.80	583.81	K8	585.28
sp|P54727|RD23B_HUMAN	ILNDDTALK	2	501.78	505.78	K8	507.26
sp|O76070|SYUG_HUMAN	EQANAVSEAVVSSVNTVATK	3	668.68	671.35	K8	676.66
sp|O76070|SYUG_HUMAN	EGVVGAVEK	2	444.25	448.25	K8	449.23
sp|O76070|SYUG_HUMAN	ENVVQSVTSVAEK	2	695.36	699.37	K8	703.34
sp|O76070|SYUG_HUMAN	TVEEAENIAVTSGVVR	2	837.44	842.44	R10	847.41
sp|P09493|TPM1_HUMAN	HIAEDADR	2	463.72	468.72	R10	470.20
sp|P09493|TPM1_HUMAN	LVIIESDLER	2	593.84	598.84	R10	600.32
sp|P09493|TPM1_HUMAN	SIDDLEDELYAQK	2	769.86	773.87	K8	777.34
sp|P09493|TPM1_HUMAN	QLEDELVSLQK	2	651.35	655.36	K8	658.33
sp|O00762|UBE2C_HUMAN	YLQETYSK	2	516.26	520.26	K8	521.24
sp|O00762|UBE2C_HUMAN	WSALYDVR	2	505.26	510.26	R10	511.24
sp|O00762|UBE2C_HUMAN	LSLEFPSGYPYNAPTVK	2	941.98	945.99	K8	951.45
sp|O00762|UBE2C_HUMAN	DPAATSVAAAR	2	515.27	520.27	R10	522.25
sp|O00762|UBE2C_HUMAN	GISAFPESDNLFK	2	712.86	716.86	K8	720.33
sp|P63279|UBC9_HUMAN	DDYPSSPPK	2	503.23	507.24	K8	508.21
sp|P63279|UBC9_HUMAN	GTPWEGGLFK	2	546.28	550.29	K8	552.26

sp|P63279|UBC9_HUMAN	DWRPAITIK	3	367.21	369.88	K8	371.87
sp|P63279|UBC9_HUMAN	DHPFGFVAVPTK	3	438.90	441.57	K8	443.88
sp|P00974|BPT1_BOVIN	AGLCQTFVYGGCR	2	744.84	747.35	V5	N/A
sp|P41160|LEP_MOUSE	INDISHTQSVSAK	3	467.24	469.92	K8	N/A
sp|P68082|MYG_HORSE	LFTGHPETLEK	3	424.56	427.23	K8	N/A
sp|P02687|MBP_BOVIN	YLASASTMDHAR	3	441.54	443.55	R6	N/A
sp|P02687|MBP_BOVIN	HGFLPR	2	363.71	366.72	R6	N/A
sp|P07288|KLK3_HUMAN	LSEPAELTDAVK	3	424.89	427.57	K8	N/A
sp|P07288|KLK3_HUMAN	IVGGWECEK	2	539.26	541.76	V5	N/A
sp|P00433|PER1A_ARMRU	SSDLVALSGGHTFGK	3	492.59	495.26	K8	N/A
sp|P02741|CRP_HUMAN	GYSIFSYATK	2	568.78	572.79	K8	N/A
sp|P02741|CRP_HUMAN	ESDTSYVSLK	2	564.77	568.78	K8	N/A

Document S1: Study 9.1 (Phase II) SOP

Study 9-1 SOP (Phase II) for NCI CPTAC Consortium-Wide Multiple Reaction Monitoring (MRM) Experiment

9-Point Calibration Curve of 123 target peptides

Experimental Design and Statistics Verification Studies Working Group

Overview

Study 9-1, the 9 point calibration curve will entail monitoring 123 signature peptides and corresponding stable-isotope labeled internal standard (SIS) peptides (~750 total transitions) that will be quantitatively assayed by time-scheduled LC-MRM-MS against a background of MARS-14 depleted human K_2EDTA plasma (0.5 μg/μL).

Aims: The study described in this SOP is designed to accomplish the following:

1. To generate a 9 point standard curve for 123 potentially cancer relevant peptide targets spanning 1 amol/μL to 100 fmol/μL in a depleted digested plasma background (500 ng/μL) that will be analyzed by LC-MRM-MS on four different triple quadrupole platforms at volunteer CPTAC sites.

2. To enable calculations of LOD, LOQ, accuracy and precision for all peptides from the calibration curve.

3. A set of 6 blinded samples is provided that mimic real biomarker samples.

4. To evaluate the level of endogenous and interfering signals for all peptides by replicate measurements of blank plasma samples.

The number of transitions that will be targeted in this method (~750) is a critical challenge and will require the use of retention time scheduled LC-MRM-MS. To achieve success, the following protocol is designed to assess the system suitability and retention time stability of the LC-MRM-MS instrument, as well as design a single MRM-MS method empirically. The protocol outlined herein should be followed as strictly as possible and all deviations from this protocol must be outlined in detail on the last sheet. The experimental design is outlined below.

Experimental Outline

1. Run System Suitability Sample 5 times to condition column and assess LC-MRM-MS performance
2. Using Skyline, create 6 MRM-MS transition lists for the heavy target peptides only (maximum of 60 transitions in each unscheduled method)
3. Analyze the heavy-only peptide sample using the 6 different MRM-MS methods
    i. Import the 6 raw files into Skyline
    ii. Obtain retention times for each heavy target peptide
    iii. Export a scheduled MRM-MS transition list (or MRM-MS method) with a 4 min RT window
    iv. Run the scheduled LC-MRM-MS method twice using the sample containing heavy peptides spiked into depleted, digested plasma.
    v. Import the 2 raw files into Skyline
    vi. Add in the light (unlabeled) peptides into the Skyline document
    vii. Export a new scheduled LC-MRM-MS method with all ~750 transitions and a 2 min RT window
4. Execute calibration curve and blinded samples according to specified run order.
5. Import data into Skyline and integrate.
    i. Import System Suitability Sample files into System Suitability Skyline file and integrate. Ensure peak area CVs and retention times are within specified error limits.
    ii. Import Calibration Curve files into Study 9-1 Skyline file and integrate.
6. Export reports from each Skyline file and upload to the NIST ftp server (see separate document).

Materials and Reagents

A. System Suitability Sample (Study 9-1-SSS)
   a. Digest of 6 equimolar proteins (Michrom Bioresources, #PTD/00001/63)
      i. Four 10 µL aliquots, 1 pmol/µL, supplied in 30% acetonitrile/0.1 % formic acid in water

B. Heavy-Only IS Peptide Mixture (in 25 fmol/uL 6 protein mix)
   a. 123 heavy IS peptides at 800 fmol/µL each
      i. Two 10 µL aliquots, supplied in 30% acetonitrile/0.1% formic acid

C. QC Sample (Sample 9-1-QC)
   a. Equimolar mixture of the 123 unlabeled and 123 labeled synthetic peptides in 25 fmol/uL 6 Protein mix matrix
      i. Four 25 µL aliquot supplied at 10 fmol/µL each peptide in 0.1% formic acid in water. Background of 25 fmol/uL of 6 protein mix added to minimize adsorption/loss of hydrophobic peptides.

D. Digested human plasma spiked with labeled IS peptides only, (Sample 9-1-A)
   a. Depleted plasma diluted to a final concentration after digestion of approximately 0.5 µg/µL
      i. 123 labeled IS peptides spiked at a concentration of 10 fmol/µL (Sample 9-1-A)
      ii. Ten 25 µL aliquots supplied in 0.1 % formic acid in water (after desalting by SPE, note: desalting was already performed at Vanderbilt University)

E. Digested human depleted plasma spiked with 123 unlabeled synthetic peptides and 123 labeled IS peptides (Samples 9-1-B to 9-1-J)
   a. Depleted plasma diluted to a final concentration after digestion of ~ 0.5 µg/µL
      i. 123 labeled IS peptides spiked at a concentration of 10 fmol/µL
      ii. Four 25 µL aliquot of each spike level supplied for 4 singlicate curves
      iii. 123 unlabeled synthetic peptides are spiked in at the following approximate concentrations:

Sample (Study 9-1)	Spiked [$^{12}C/^{14}N$] peptide Concentration (fmol/µL)
9-1-J	100.00
9-1-I	23.71
9-1-H	5.62
9-1-G	1.33
9-1-F	0.316
9-1-E	0.075
9-1-D	0.018
9-1-C	0.004
9-1-B	0.001

Important note: each of the 4 different singlicate calibration curves will be completed before the next replicate curve will be started: Specific naming including the first blank (A sample) is required, for details also see run order below:
9-1-A1 to 9-1-J1; 9-1-A2 to 9-1-J2; 9-1-A3 to 9-1-J3; 9-1-A4 to 9-1-J4.

F. Six Blinded Samples to be run in four replicates in between each singlicate calibration curve (Total: Samples 9-1-01Blinded to 9-1-24Blinded)

Four replicates of 6 Blinded samples (each provided in 25 aliquot):

first replicate:	9-1-01Blinded	to	9-1-06Blinded
second replicate:	9-1-07Blinded	to	9-1-12Blinded
third replicate:	9-1-13Blinded	to	9-1-18Blinded
fourth replicate:	9-1-19Blinded	to	9-1-24Blinded

mixed run order for the Blinded Samples within each replicate set

G. "Wash Samples", to avoid carryover between blinded samples, the run order defines running a "wash" gradient in between blinded samples. "**Wash Samples**" will be 10 fmol/uL of the 6 protein mix (Michrom sample). Samples will be diluted from provided 6 protein mix / Michrom sample at stock concentration of 1 pmol/µL).

H. HPLC solvents

a. Acetonitrile, HPLC grade
b. Water, HPLC grade
c. Formic Acid

I. PicoFrit columns

a. 75 µm ID with 10 µm ID tip
b. For ABSciex and Thermo operators: Prepacked New Objective Reprosil Columns that were sent to all laboratories from Vanderbilt University (in case the operator runs out of prepacked columns please contact Susan Abbatiello or Birgit Schilling)
c. For Waters UPLC operators: a Waters specific column will be used (1.7µm BEH130 C18, 75um X 150mm column)

J. Loop for Eksigent LC: 1 µL PEEKsil loop provided in sample kit, 100 µm ID PEEKsil; newer design 1 µL loops are provided for Eksigent Ultra systems.

K. Fused silica

a. 20 µm ID between gradient pump and autosampler (for direct inject configuration)
b. 20 µm ID between autosampler and PicoFrit column (keep tubing as short as possible)

L. High voltage contact

a. For liquid-liquid junction, Upchurch part P-888.

M. Autosampler vials

a. Polypropylene autosampler vials with conical insert, 250 µL maximum volume

N. Autosampler loop

a. 1 µL loop for direct inject set-up (fused silica or PEEKsil)

O. Column heater

a. If the system is equipped with a column heater, please set to 35 degrees Celsius.

Required Software

A. **Skyline Daily Software**, most recent version (please note version used)
B. **Platform-specific Skyline document** (*.sky), provided by CPTAC VWG
C. **Microsoft Excel** (or similar)
D. **AuDIT Software** (available at http://www.broadinstitute.org/cancer/software/genepattern/modules/AuDIT.html)

Overview

In Main Study 9-1 (9-point calibration curve), synthetic [$^{12}C/^{14}N$] and [$^{13}C/^{15}N$] signature peptides will be spiked into MARS-14 depleted, digested human K_2EDTA plasma and analyzed by scheduled LC-MRM-MS at all sites. All sample preparation will be performed at Vanderbilt University prior to distribution of the sample kits. Results from Study 9-1 from all CPTAC sites, will provide LOD/LOQ values for each peptide (this design contains minimal sample handling, and no target protein digestion and eliminates key factors of experimental variability). The Study 9-1 scheduled LC-MRM-MS samples will consist of a 9-point standard curve ranging in concentration from 100 fmol/µL to 1 amol/µL, with all concentrations in the presence of 0.5 µg/µL depleted digested plasma. Synthetic signature internal standard (IS) peptides uniformly labeled with a $^{13}C/^{15}N$ amino acid will be spiked into all plasma samples at a constant concentration of 10 fmol/µL.

The following paragraphs provide a description of the experimental procedure, with each major step separated into its own paragraph and heading. Paragraphs are followed by outlined "checklists" that have matching headings for reference.

1. System Suitability Sample, Column Conditioning and Instrument Performance

System Suitability Sample (SSS) runs using the Michrom 6 protein mix (based on Study 9S) will be interspersed into Study 9-1 (to guarantee system suitability and performance and, in particular, monitor peak area stability and potential RT drift). The SSS will first be analyzed in an unscheduled LC-MRM-MS method (Study 9S) to condition the column and assess the performance of the LC-MS instrument platform. Upon obtaining the specified results for retention time and peak area, sites may proceed to the generation of the scheduled LC-MRM-MS runs for Study 9.

The SSS will also be run every 6-8 runs in the sample queue, in order to track instrument performance. Data will be analyzed in Skyline and will be submitted in a separate "Michrom Study 9S" file.

2. Generation of Scheduled LC-MRM-MS Method for Study 9 Peptides

In preparation for the scheduled calibration curve LC-MRM-MS runs, in which 738 transitions will be monitored in one run, participating sites will monitor all heavy synthetic labeled peptides with about 369 transitions (123 peptides with 3 transitions each) in six unscheduled LC-MRM-MS runs (~60 transitions per run), with each run represented by a separate LC-MRM-MS method. Retention times for scheduling will be determined empirically using these six runs and will be verified by analysis with a single scheduled LC-MRM-MS run prior to analyzing the samples from which the calibration curve will be generated. All method building and data analysis will be performed using Skyline.

3. Data Analysis

The experimentally determined molar concentration of the spiked peptide or protein will be calculated and compared to its theoretical value for accuracy. Operators will import all acquired data files into Skyline and check and if necessary adjust peak integration. Check "Integrate All" (Menu, Settings, Integrate all), so that heavy and light transitions will be "integrated together".

While checking peak integration open RT replicate views (Menu, View, Retention Time, Replicate Comparison), and Peak Area replicate views (Menu, View, Peak Areas, Replicate Comparison) to visually help confirm proper peak integration. Specifically, check for interferences and RT drift problems during scheduling. Use Skyline Custom Annotation features to annotate any observations or notes for Study Statisticians to consider (Menu, View, Results Grid; and annotate observations on the precursor or transition level). Finally use "AuDIT" to analyze data for interferences before submitting data to statisticians. Linear plots of response versus known concentration from each of the 9-point standard curves will be used to evaluate the linearity of the MRM measurements across the range of spiked peptide concentrations, thus providing evidence of a quantitative measurement process. Replicate analyses of the spiked plasma samples will provide estimates of assay precision (standard deviation and % CV), plus LOQ and LOD will be determined at defined signal-to-noise ratio (S/N) values. Blank runs of digested plasma with labeled peptides will provide estimates of chemical background levels in the absence of unlabeled signature peptide peaks as well as the presence of endogenous peptides in the sample (*i.e.*, CRP). Furthermore, an estimate of carryover will be determined by running a series of gradient HPLC washout runs. Finally, variation across CPTAC sites will be assessed for each of these characteristic analytical metrics.

4. Troubleshooting

This study contains a large number of samples that need to be run in a defined order and requires very reproducible peptide RTs. Problems may arise that will affect RT stability, including, but not limited to, increased column pressure, tip blockage, significant change in ambient temperature or injection of air into the system. The troubleshooting section provides some suggested routes of diagnosis and an outline of how to resolve the problem and continue with data acquisition. Importantly, operators are instructed to use the SSS and Skyline to observe any problems with retention time shifting, asymmetric peaks, low signal, and asymmetric peak shape. These problems can be caught and rectified without much down time and with minimal re-running of sample.

Procedures

1. **System Suitability Sample – Column Conditioning**
    a. Sample preparation
        i. Dilute SSS from 1 pmol/µL to 50 fmol/µL
            1. Add 95 µL of 0.1% formic acid/3% acetonitrile to an Eppendorf tube
            2. Add 5 µL of MichromMix (SSS) to the vial and vortex
            3. Centrifuge sample for 1 min in a benchtop centrifuge
            4. Transfer to an autosampler vial and place in autosampler
    b. Method Preparation
        i. Prepare SSS transition list from the appropriate Skyline file
            1. 115 transitions, 22 peptides
            2. Q1, Q3 resolution = unit
            3. Dwell time = 10 msec
            4. Interscan delay (where applicable) = 5 msec (3 msec for AB Sciex 5500)
        ii. Use the **Study 9S** LC gradient (also see Table 2b)
            1. 0-5 min, 3% B; 5-8 min, 3-15% B; 8-42 min 15-35% B; 42-45 min, 35-90% B, 45-49 min, 90% B hold; 49-50 min, 90-3% B; 50-80 min, 3% B. *Note: operators with Waters Aquity UPLC will use a slighly adjusted gradient (due to the use of Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC, see Table 3b).*
            2. Flow rate: 300 nL/min
            3. Mobile phase A: 0.1% formic acid in water (v/v); Mobile phase B: 90% acetonitrile (v/v)/0.1% formic acid in water (v/v). *Note: operators with Waters Aquity UPLC will use Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC).*
        iii. Autosampler Settings
            1. Direct Injection, full-loop injection, 1 µL sample loop
            2. Pick up 2 µL of sample sandwiched between mobile phase A (0.1% formic acid)
            3. See Table 1 for example
        iv. Column heater Settings
            1. If using a column heater, please set to 35 degrees Celsius.
        v. Recommended MS Source Conditions
            1. AB Sciex (4000 QTRAP and QTRAP 5500)
                a. ESI voltage: 2200 ± 200 V ; Curtain gas: 20 ± 5; GS1: 5 ± 5; IHT: 150 ˚C
            2. ThermoFisher Scientific (Vantage)
                b. ESI voltage: 1300-1400 V; Declustering voltage: -1 V; Capillary Temperature: 210 ˚C
            3. Waters Xevo
                c. Capillary voltage: 2800 V; Cone voltage: 35V; Ion source temperature: 150 ˚C; Cone gas flow: 15 L/Hr; Nanoflow gas flow: 0.20 bar
            4. Agilent 6410/6460

d. ESI voltage: 1700-1800 V; gas flow: 2.5 L/min; temperature: 325˚C; fragmentor voltage: 125 V

For all instruments:
Q1, Q3 resolution = unit
Dwell time = 10 msec
Interscan delay (where applicable) = 5 msec (3 msec for AB Sciex 5500)

c. Inject sample 5 times
d. Data Analysis
   i. Import data files into Skyline System Suitability Sample file
   ii. Check automatic integration of all peaks
      1. Manually adjust integration of peaks, if necessary
      2. Make sure integration start and stop is identical for all transitions of a precursor (go to "Settings", and check "Integrate All" to enable this feature automatically)
      3. Document any peak that looks problematic by utilizing the "note" feature in Skyline
         a. Excessive tailing or fronting
         b. Drop-out of electrospray
         c. Poor peak shape
         d. Missing transitions
   iii. Check that peaks pass criteria
      1. RT shift is isolated to first 3 injections
      2. Peak area CV is less than 30% for all peaks
      3. All peaks are detected in the last 2 sample runs
   iv. If data do not pass criteria, troubleshoot the LC system and re-run SSS column conditioning procedure
   v. If necessary, contact CPTAC VWG members for advice or assistance
      1. Susan Abbatiello, susana@broadinstitute.org
      2. Birgit Schilling, bschilling@buckinstitute.org

2. <u>Retention Time Scheduling for Heavy Peptides</u>

a. Sample preparation
   i. Dilute Heavy-Only IS peptide mixture from 800 fmol/µL to 80 fmol/µL
      1. Add 45 µL 3% acetonitrile/0.1% formic acid to an Eppendorf tube
      2. Add 5 µL of the 800 fmol/mL Heavy-Only IS peptide stock to the vial, vortex
      3. Transfer to an autosampler vial and place in autosampler
b. Method Preparation
   i. Prepare the Heavy-Only IS peptide transition list from the appropriate Skyline file
      1. 369 transitions, 123 peptides, 123 precursors
      2. Q1, Q3 resolution = unit
      3. Dwell time = 10 msec (can be lower for AB Sciex 5500 instrument, minimum is 5 msec)
      4. Interscan delay (where applicable) = 5 msec (3 msec for AB Sciex 5500)
      5. Maximum number of concurrent transitions: 60
      6. Export transition list as multiple methods, ignore proteins

ii. Use the **Study 9-1** LC gradient (also see Table 2a)
    1. 0-5 min, 3% B; 5-8 min, 3-7% B; 8-35 min 7-25% B; 35-42 min, 25-40% B, 42-45 min, 40-90% B; 45-49 min, 90% B hold; 49-50 min, 90-3% B; 50-80 min, 3% B. *Note: operators with Waters Aquity UPLC will use a slighly adjusted gradient (due to the use of Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC, see Table 3a).*
    2. Flow rate: 300 nL/min
    3. Mobile phase A: 0.1% formic acid in water (v/v); Mobile phase B: 90% acetonitrile (v/v)/0.1% formic acid in water (v/v). *Note: operators with Waters Aquity UPLC will use Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC).*

iii. Autosampler Settings
    1. Direct Injection, full-loop injection, 1 µL sample load
    2. Pick up 2 µL of sample sandwiched between mobile phase A (0.1% formic acid)
    3. See Table 1 for example

iv. Recommended MS Source Conditions (use as guidelines, see above, Section 1.b.iv.)

c. Inject the sample 6 times, one time for each method created in Skyline
    i. Name each injection with its own filename/samplename so that each injection is its own, individual file
        1. Study9-1_SiteXX_HeavyOnly_method01
        2. Study9-1_SiteXX_HeavyOnly_method02
        3. Study9-1_SiteXX_HeavyOnly_method03
        4. Study9-1_SiteXX_HeavyOnly_method04
        5. Study9-1_SiteXX_HeavyOnly_method05
        6. Study9-1_SiteXX_HeavyOnly_method06
    ii. Follow with 1-5 injections of SSS
        1. Do not allow the system to sit idle after the 6 HeavyOnly sample runs

d. Data Analysis
    i. Import data files into the appropriate Skyline Platform-specific Study 9-1 file
        1. Import multiple files as one replicate
        2. Name replicate (HeavyOnly_rep1)
    ii. Check automatic integration of all peaks
        1. Manually adjust integration of peaks, if necessary
        2. Make sure integration start and stop is identical for all transitions of a precursor (check integrate all in Skyline)
        3. Document any peak that looks problematic using the "note" feature in Skyline - Use Skyline **Custom Annotation** to note any of the above features as described below (see separate document)
            a. Excessive tailing or fronting
            b. Drop-out of electrospray
            c. Poor peak shape
            d. Missing transitions

iii. Under Peptide Settings, set window to 4 minutes
    1. Export the transition list in scheduled mode – heavy only, ~325 transitions with 4 min RT window. Double check that all 325 transitions are exported and added into the MS method appropriately.
    2. Run two replicates of Study 9-1 A and import into Skyline
    3. Ensure all peaks are detected
iv. Under Peptide Settings, set RT window to 2 minutes.
v. Under Edit->Refine, add light labeled peptides to the document
    1. There should now be 738 transitions, 123 peptides, 246 precursors
    2. View the RT plot for Scheduling
    3. Ensure the maximum number of concurrent transitions throughout the gradient is < 80
    4. If there are incidents of concurrent transitions > 80 anywhere in the gradient, contact CPTAC VWG members for assistance

e. Export all transitions (~750) in Scheduled mode, with 2 min RT window. Double check that all 738 transitions are exported and added into the MS method appropriately.
f. Acquire 4 replicates of sample Study9-1 A with the scheduled LC-MRM-MS method
    i. Import data back into Skyline and observe any shift in retention times
    ii. Use RT graph, peptide replicate view
    iii. If peaks shift by > 30 seconds (0.5 minutes), do not proceed and contact CPTAC VWG members for assistance
    iv. Look at data to make sure peak apexes are in or near the center of the RT window and not getting cut off during detection.
g. Continue acquiring Study9-1 samples according to the sample chart shown below.

Important Note:
Different than in Study 7, the four replicate concentration points will be acquired in **4x singlicate curves** (with blanks and SSS in between to avoid carryover). Also note each singlicate concentration point was prepared individually at the central preparation site (Vanderbilt), so for each concentration point there will be 4 tubes supplied.

Adding Calibration Curve samples and Blinded Samples into Autosampler Vials:
Add one Calibration Curve and corresponding Blinded Samples at a time into the Autosampler. Each Calibration Curve concentration point tube contains 25 µl sample, for each singlicate curve remove the appropriate singlicate aliquot tube from the freezer, fill 12 µl into an autosampler vial, and store the remainder of that aliquot in the +4 degrees centigrade refrigerator (to avoid additional freeze-thaw cycles). Make sure to finish each singlicate curve (including initial data analysis and review of data points for quality, or for retention time drift of peaks outside the RT scheduling window) before starting the next singlicate curve. If data points need to be rerun from the current singlicate curve use remaining sample that is still in autosampler vials or if multiple reruns are needed use the saved other half of the specific singlicate aliquot that was transferred into the refrigerator as described above. For the 9-1-A sample used as blank (A-sample) at the beginning of a singlicate curve, and as blank in between blinded samples fill the entire A aliquot into an autosampler vial.

In Case of Acquisition Problems:
In case there are problems with the data acquisition or RT drift, and possible reruns that may become necessary, please keep the run number at the end of the filename to keep track of the run order. The run number should agree with the chronological order in which the samples were analyzed by LC-MRM-MS.

Blinded Samples:
Six blinded samples will be run with 1 wash run (10 fmol 6 protein mix) and 1 blank run (heavy peptides in plasma matrix, 9-1-A) in between. A total of 4 replicates of the 6 blinded samples will be acquired, one set of six in between each of the four singlicate calibration curves yielding a total of 24 blinded samples (Samples 9-1-01Blinded to 9-1-24Blinded). The wash runs use a different wash gradient **9-1-Wash** (see Table 2c below).
For Blinded Samples use Study 9-1 gradient (Table 2a) and "Study 9 scheduled MRM-MS, light and heavy" MS-method, for Wash Samples use Wash gradient (Table 2c) and "Study 9S, SSS"-adjusted MS-method, and for Blank Samples use Study 9-1 gradient (Table 2a) and "Study 9 scheduled MRM-MS, light and heavy" MS-method. Blanks (in between blinded samples) can be injected from the same autosampler vial.

Fresh Preparation of System Suitability samples (SSS) for each Singlicate Curve:
For each Singlicate Calibration Curve a fresh autosampler vial of the SSS will be prepared to prevent degradation of the sample (note in a notebook when fresh SSS are added).

Please adhere closely to sample naming as defined in the run order (with chronological runorder number at the end).

Please remember: there are 3 different gradients to be used in the below run orders:

- **Study 9S** gradient for System Suitability Samples
- **Study 9-1** gradient for the main study (all concentration curve points as well as blinded samples)
- **Study 9 wash** gradient (in between blinded samples)

Gradients are described in detail in Tables 2a-c, and 3a-c for Waters UPLC (see below)

First singlicate curve plus blinded samples (01-06blinded)

Run Number	Number of injections	Sample Description	Filename	Method	Notes
1	1	SSS, Michrom Mix	9-1_SiteX_SSS_run_001	Study 9S (SSS)	Column Conditioning
2	1	SSS, Michrom Mix	9-1_SiteX_SSS_run_002	Study 9S (SSS)	
3	1	SSS, Michrom Mix	9-1_SiteX_SSS_run_003	Study 9S (SSS)	
4	1	SSS, Michrom Mix	9-1_SiteX_SSS_run_004	Study 9S (SSS)	
5	1	SSS, Michrom Mix	9-1_SiteX_SSS_run_005	Study 9S (SSS)	
6	1	Study9-1 Heavy Only	9-1_SiteX_heavy_method01_run_006	Study 9 method 01	For scheduling
7	1	Study9-1 Heavy Only	9-1_SiteX_heavy_method02_run_007	Study 9 method 02	
8	1	Study9-1 Heavy Only	9-1_SiteX_heavy_method03_run_008	Study 9 method 03	
9	1	Study9-1 Heavy Only	9-1_SiteX_heavy_method04_run_009	Study 9 method 04	
10	1	Study9-1 Heavy Only	9-1_SiteX_heavy_method05_run 010	Study 9 method 05	
11	1	Study9-1 Heavy Only	9-1_SiteX_heavy_method06_run_011	Study 9 method 06	
12-16	5	SSS, Michrom Mix	9-1_SiteX_SSS_run_012 9-1_SiteX_SSS_run_013 9-1_SiteX_SSS_run_014 9-1_SiteX_SSS_run_015 9-1_SiteX_SSS_run_016	Study 9S (SSS)	At least 3 SSS samples and if necessary place holder
17	1	Study9-1 A, IS peptides in plasma	9-1_SiteX_A_sMRM_run_017	Study 9 scheduled MRM-MS, heavy only	Scheduled run, 4 min RT window
18	1	Study9-1 A, IS peptides in plasma	9-1_SiteX_A_sMRM_run_018	Study 9 scheduled MRM-MS, heavy only	
19	1	Study9-1 A1, IS peptides in plasma	9-1_SiteX_A1_CalCurve_run_019	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window, Calibration Curve
20	1	Study9-1 B1	9-1_SiteX_B1_CalCurve_run_020	Study 9 scheduled MRM-MS, light and heavy	
21	1	Study9-1 C1	9-1_SiteX_C1_CalCurve_run_021	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
22	1	Study9-1 D1	9-1_SiteX_D1_CalCurve_run_022	Study9 sMRM	CalCurve
23	1	SSS	9-1_SiteX_SSS_run_023	Study 9S (SSS)	System Suitability
24	1	Study9-1 E1	9-1_SiteX_E1_CalCurve_run_024	Study9 sMRM	CalCurve

25	1	Study9-1 F1	9-1_SiteX_F1_CalCurve_run_025	Study9 sMRM	CalCurve
26	1	Study9-1 G1	9-1_SiteX_G1_CalCurve_run_026	Study9 sMRM	CalCurve
27	1	Study9-1 H1	9-1_SiteX_H1_CalCurve_run_027	Study9 sMRM	CalCurve
28	1	Study9-1 QC1	9-1_SiteX_QC1_CalCurve_run_028	Study9 sMRM	QC
29	1	Study9-1 I1	9-1_SiteX_I1_CalCurve_run_029	Study9 sMRM	CalCurve
30	1	Study9-1 J1	9-1_SiteX_J1_CalCurve_run_030	Study9 sMRM	CalCurve
31	1	Study9-1 wash	9-1_SiteX_wash_run_31	Study 9 wash	
32	1	SSS	9-1_SiteX_SSS_run_032	Study 9S (SSS)	System suitability
33	1	SSS	9-1_SiteX_SSS_run_033	Study 9S (SSS)	System suitability
34	1	SSS	9-1_SiteX_SSS_run_034	Study 9S (SSS)	System suitability
35	1	Study9-1 A, IS peptides in plasma	9-1_SiteX_A_blank_run_035	Study 9 scheduled MRM-MS, light and heavy	
36	1	Study9-1 01blinded-Sample	9-1_SiteX_01blinded_run_036	Study 9 scheduled MRM-MS, light and heavy	Blinded
37	1	Study9-1 wash	9-1_SiteX_wash_run_037	Study 9 wash	
38	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_038	Study 9 scheduled MRM-MS, light and heavy	
39	1	Study9-1 02blinded	9-1_SiteX_02blinded_run_039	Study 9 scheduled MRM-MS, light and heavy	Blinded
40	1	Study9-1 wash	9-1_SiteX_wash_run_040	Study 9 wash	
41	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_041	Study 9 scheduled MRM-MS, light and heavy	
42	1	SSS	9-1_SiteX_SSS_run_042	Study 9S (SSS)	System suitability
43	1	Study9-1 03blinded	9-1_SiteX_03blinded_run_043	Study 9 scheduled MRM-MS, light and heavy	Blinded
44	1	Study9-1 wash	9-1_SiteX_wash_run_044	Study 9 wash	

45	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_045**	**Study 9 scheduled MRM-MS, light and heavy**	
46	**1**	**Study9-1 04blinded**	**9-1_SiteX_04blinded_run_046**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
47	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_047**	**Study 9 wash**	
48	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_048**	**Study 9 scheduled MRM-MS, light and heavy**	
49	**1**	**SSS**	**9-1_SiteX_SSS_run_049**	**Study 9S (SSS)**	**System suitability**
50	**1**	**Study9-1 05blinded**	**9-1_SiteX_05blinded_run_050**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
51	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_051**	**Study 9 wash**	
52	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_052**	**Study 9 scheduled MRM-MS, light and heavy**	
53	**1**	**Study9-1 06blinded**	**9-1_SiteX_06blinded_run_053**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
54	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_054**	**Study 9 wash**	
55	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_055**	**Study 9 scheduled MRM-MS, light and heavy**	
			Run second, third and fourth singlicate curves (see below)		

Second singlicate curve plus blinded samples (07-12blinded)

56-58	3	SSS, Michrom Mix	9-1_SiteX_SSS_run_056 9-1_SiteX_SSS_run_057 9-1_SiteX_SSS_run_058	Study 9S (SSS)	3 SSS samples
59	1	Study9-1 A2, IS peptides in plasma	9-1_SiteX_A2_CalCurve_run_059	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window, Calibration Curve
60	1	Study9-1 B2	9-1_SiteX_B2_CalCurve_run_060	Study 9 scheduled MRM-MS, light and heavy	
61	1	Study9-1 C2	9-1_SiteX_C2_CalCurve_run_061	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
62	1	Study9-1 D2	9-1_SiteX_D2_CalCurve_run_062	Study9 sMRM	CalCurve
63	1	SSS	9-1_SiteX_SSS_run_063	Study 9S (SSS)	System Suitability
64	1	Study9-1 E2	9-1_SiteX_E2_CalCurve_run_064	Study9 sMRM	CalCurve
65	1	Study9-1 F2	9-1_SiteX_F2_CalCurve_run_065	Study9 sMRM	CalCurve
66	1	Study9-1 G2	9-1_SiteX_G2_CalCurve_run_066	Study9 sMRM	CalCurve
67	1	Study9-1 H2	9-1_SiteX_H2_CalCurve_run_067	Study9 sMRM	CalCurve
68	1	Study9-1 QC2	9-1_SiteX_QC2_CalCurve_run_068	Study9 sMRM	QC
69	1	Study9-1 I2	9-1_SiteX_I2_CalCurve_run_069	Study9 sMRM	CalCurve
70	1	Study9-1 J2	9-1_SiteX_J2_CalCurve_run_070	Study9 sMRM	CalCurve
71	1	Study9-1 wash	9-1_SiteX_wash_run_071	Study 9 wash	
72	1	SSS	9-1_SiteX_SSS_run_072	Study 9S (SSS)	System suitability
73	1	SSS	9-1_SiteX_SSS_run_073	Study 9S (SSS)	System suitability
74	1	SSS	9-1_SiteX_SSS_run_074	Study 9S (SSS)	System suitability
75	1	Study9-1 A, IS peptides in plasma	9-1_SiteX_A_blank_run_075	Study 9 scheduled MRM-MS, light and heavy	
76	1	Study9-1 07blinded-Sample	9-1_SiteX_07blinded_run_076	Study 9 scheduled MRM-MS, light and heavy	Blinded
77	1	Study9-1 wash	9-1_SiteX_wash_run_077	Study 9 wash	

78	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_078**	**Study 9 scheduled MRM-MS, light and heavy**	
79	**1**	**Study9-1 08blinded**	**9-1_SiteX_08blinded_run_079**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
80	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_080**	**Study 9 wash**	
81	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_081**	**Study 9 scheduled MRM-MS, light and heavy**	
82	**1**	**SSS**	**9-1_SiteX_SSS_run_082**	**Study 9S (SSS)**	**System suitability**
83	**1**	**Study9-1 09blinded**	**9-1_SiteX_09blinded_run_083**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
84	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_084**	**Study 9 wash**	
85	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_085**	**Study 9 scheduled MRM-MS, light and heavy**	
86	**1**	**Study9-1 10blinded**	**9-1_SiteX_10blinded_run_086**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
87	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_087**	**Study 9 wash**	
88	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_088**	**Study 9 scheduled MRM-MS, light and heavy**	
89	**1**	**SSS**	**9-1_SiteX_SSS_run_089**	**Study 9S (SSS)**	**System suitability**
90	**1**	**Study9-1 11blinded**	**9-1_SiteX_11blinded_run_090**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
91	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_091**	**Study 9 wash**	
92	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_092**	**Study 9 scheduled MRM-MS, light and heavy**	

93	1	Study9-1 12blinded	9-1_SiteX_12blinded_run_093	Study 9 scheduled MRM-MS, light and heavy	Blinded
94	1	Study9-1 wash	9-1_SiteX_wash_run_094	Study 9 wash	
95	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_095	Study 9 scheduled MRM-MS, light and heavy	
			Run third and fourth singlicate curves (see below)		

Third singlicate curve plus blinded samples (13-18blinded)

96-98	3	SSS, Michrom Mix	9-1_SiteX_SSS_run_096 9-1_SiteX_SSS_run_097 9-1_SiteX_SSS_run_098	Study 9S (SSS)	3 SSS samples
99	1	Study9-1 A3, IS peptides in plasma	9-1_SiteX_A3_CalCurve_run_099	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window, Calibration Curve
100	1	Study9-1 B3	9-1_SiteX_B3_CalCurve_run_100	Study 9 scheduled MRM-MS, light and heavy	
101	1	Study9-1 C3	9-1_SiteX_C3_CalCurve_run_101	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
102	1	Study9-1 D3	9-1_SiteX_D3_CalCurve_run_102	Study9 sMRM	CalCurve
103	1	SSS	9-1_SiteX_SSS_run_103	Study 9S (SSS)	System Suitability
104	1	Study9-1 E3	9-1_SiteX_E3_CalCurve_run_104	Study9 sMRM	CalCurve
105	1	Study9-1 F3	9-1_SiteX_F3_CalCurve_run_105	Study9 sMRM	CalCurve
106	1	Study9-1 G3	9-1_SiteX_G3_CalCurve_run_106	Study9 sMRM	CalCurve
107	1	Study9-1 H3	9-1_SiteX_H3_CalCurve_run_107	Study9 sMRM	CalCurve
108	1	Study9-1 QC3	9-1_SiteX_QC3_CalCurve_run_108	Study9 sMRM	QC
109	1	Study9-1 I3	9-1_SiteX_I3_CalCurve_run_109	Study9 sMRM	CalCurve
110	1	Study9-1 J3	9-1_SiteX_J3_CalCurve_run_110	Study9 sMRM	CalCurve

111	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_111**	**Study 9 wash**	
112	**1**	**SSS**	**9-1_SiteX_SSS_run_112**	**Study 9S (SSS)**	**System suitability**
113	**1**	**SSS**	**9-1_SiteX_SSS_run_113**	**Study 9S (SSS)**	**System suitability**
114	**1**	**SSS**	**9-1_SiteX_SSS_run_114**	**Study 9S (SSS)**	**System suitability**
115	**1**	**Study9-1 A, IS peptides in plasma**	**9-1_SiteX_A_blank_run_115**	**Study 9 scheduled MRM-MS, light and heavy**	
116	**1**	**Study9-1 13blinded-Sample**	**9-1_SiteX_13blinded_run_116**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
117	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_117**	**Study 9 wash**	
118	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_118**	**Study 9 scheduled MRM-MS, light and heavy**	
119	**1**	**Study9-1 14blinded**	**9-1_SiteX_14blinded_run_119**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
120	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_120**	**Study 9 wash**	
121	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_121**	**Study 9 scheduled MRM-MS, light and heavy**	
122	**1**	**SSS**	**9-1_SiteX_SSS_run_122**	**Study 9S (SSS)**	**System suitability**
123	**1**	**Study9-1 15blinded**	**9-1_SiteX_15blinded_run_123**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
124	**1**	**Study9-1 wash**	**9-1_SiteX_wash_run_124**	**Study 9 wash**	
125	**1**	**IS peptides in plasma, blank**	**9-1_SiteX_A_blank _run_125**	**Study 9 scheduled MRM-MS, light and heavy**	
126	**1**	**Study9-1 16blinded**	**9-1_SiteX_16blinded_run_126**	**Study 9 scheduled MRM-MS, light**	**Blinded**

				and heavy	
127	1	Study9-1 wash	9-1_SiteX_wash_run_127	Study 9 wash	
128	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_128	Study 9 scheduled MRM-MS, light and heavy	
129	1	SSS	9-1_SiteX_SSS_run_129	Study 9S (SSS)	System suitability
130	1	Study9-1 17blinded	9-1_SiteX_17blinded_run_130	Study 9 scheduled MRM-MS, light and heavy	Blinded
131	1	Study9-1 wash	9-1_SiteX_wash_run_131	Study 9 wash	
132	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_132	Study 9 scheduled MRM-MS, light and heavy	
133	1	Study9-1 18blinded	9-1_SiteX_18blinded_run_133	Study 9 scheduled MRM-MS, light and heavy	Blinded
134	1	Study9-1 wash	9-1_SiteX_wash_run_134	Study 9 wash	
135	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_135	Study 9 scheduled MRM-MS, light and heavy	
			Run fourth singlicate curve (see below)		

Fourth singlicate curve plus blinded samples (19-24blinded)

136-138	3	SSS, Michrom Mix	9-1_SiteX_SSS_run_136 9-1_SiteX_SSS_run_137 9-1_SiteX_SSS_run_138	Study 9S (SSS)	3 SSS samples
139	1	Study9-1 A4, IS peptides in plasma	9-1_SiteX_A4_CalCurve_run_139	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window, Calibration Curve
140	1	Study9-1 B4	9-1_SiteX_B4_CalCurve_run_140	Study 9 scheduled MRM-MS, light and heavy	

141	1	Study9-1 C4	9-1_SiteX_C4_CalCurve_run_141	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
142	1	Study9-1 D4	9-1_SiteX_D4_CalCurve_run_142	Study9 sMRM	CalCurve
143	1	SSS	9-1_SiteX_SSS_run_143	Study 9S (SSS)	System Suitability
144	1	Study9-1 E4	9-1_SiteX_E4_CalCurve_run_144	Study9 sMRM	CalCurve
145	1	Study9-1 F4	9-1_SiteX_F4_CalCurve_run_145	Study9 sMRM	CalCurve
146	1	Study9-1 G4	9-1_SiteX_G4_CalCurve_run_146	Study9 sMRM	CalCurve
147	1	Study9-1 H4	9-1_SiteX_H4_CalCurve_run_147	Study9 sMRM	CalCurve
148	1	Study9-1 QC4	9-1_SiteX_QC4_CalCurve_run_148	Study9 sMRM	QC
149	1	Study9-1 I4	9-1_SiteX_I4_CalCurve_run_149	Study9 sMRM	CalCurve
150	1	Study9-1 J4	9-1_SiteX_J4_CalCurve_run_150	Study9 sMRM	CalCurve
151	1	Study9-1 wash	9-1_SiteX_wash_run_151	Study 9 wash	
152	1	SSS	9-1_SiteX_SSS_run_152	Study 9S (SSS)	System suitability
153	1	SSS	9-1_SiteX_SSS_run_153	Study 9S (SSS)	System suitability
154	1	SSS	9-1_SiteX_SSS_run_154	Study 9S (SSS)	System suitability
155	1	Study9-1 A, IS peptides in plasma	9-1_SiteX_A_blank_run_155	Study 9 scheduled MRM-MS, light and heavy	
156	1	Study9-1 19blinded-Sample	9-1_SiteX_19blinded_run_156	Study 9 scheduled MRM-MS, light and heavy	Blinded
157	1	Study9-1 wash	9-1_SiteX_wash_run_157	Study 9 wash	
158	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_158	Study 9 scheduled MRM-MS, light and heavy	
159	1	Study9-1 20blinded	9-1_SiteX_20blinded_run_159	Study 9 scheduled MRM-MS, light and heavy	Blinded
160	1	Study9-1 wash	9-1_SiteX_wash_run_160	Study 9 wash	
161	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_161	Study 9 scheduled	

				MRM-MS, light and heavy	
162	1	SSS	9-1_SiteX_SSS_run_162	Study 9S (SSS)	System suitability
163	1	Study9-1 21blinded	9-1_SiteX_21blinded_run_163	Study 9 scheduled MRM-MS, light and heavy	Blinded
164	1	Study9-1 wash	9-1_SiteX_wash_run_164	Study 9 wash	
165	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_165	Study 9 scheduled MRM-MS, light and heavy	
166	1	Study9-1 22blinded	9-1_SiteX_22blinded_run_166	Study 9 scheduled MRM-MS, light and heavy	Blinded
167	1	Study9-1 wash	9-1_SiteX_wash_run_167	Study 9 wash	
168	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_168	Study 9 scheduled MRM-MS, light and heavy	
169	1	SSS	9-1_SiteX_SSS_run_169	Study 9S (SSS)	System suitability
170	1	Study9-1 23blinded	9-1_SiteX_23blinded_run_170	Study 9 scheduled MRM-MS, light and heavy	Blinded
171	1	Study9-1 wash	9-1_SiteX_wash_run_171	Study 9 wash	
172	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_172	Study 9 scheduled MRM-MS, light and heavy	
173	1	Study9-1 24blinded	9-1_SiteX_24blinded_run_173	Study 9 scheduled MRM-MS, light and heavy	Blinded
174	1	Study9-1 wash	9-1_SiteX_wash_run_174	Study 9 wash	
175	1	IS peptides in plasma, blank	9-1_SiteX_A_blank _run_175	Study 9 scheduled MRM-MS, light and heavy	
			Study 9-1finished !!		

1. **Data Analysis**
    a. All data should be analyzed in "real time," or as close to its acquisition time as possible
    b. Data analysis must be conducted through Skyline using templates provided for each instrument platform
    c. Once data are imported, check integration of all peaks
        i. Integrate transitions with the same start and stop time: Under "Settings" in Skyline, enable "Integrate All"
        ii. Integrate light and heavy peptides with the same start and stop time
        iii. If an interference is present, document the transition-level note for that transition
    d. Export data for submission to VWG statisticians using the report template provided
    e. Under Settings, click on "custom annotation" and make sure that all settings are selected.
        i. If a transition, precursor, or peptide is deemed unusable because of a bad injection, loss of electrospray or another explainable reason and the sample is re-run, please click the "do not use" box for the sample that is to be excluded from subsequent analysis for LOD/LOQ.

Even if a specific replicate needs to be rerun, the original and new (rerun) replicate should both be imported into Skyline, and "custom annotation" capabilities of Skyline should be used to annotate what data points should be used for statistical analysis.

4. **Trouble Shooting Section**
    a. **Symptom: No data (missing peaks) or poor quality peaks for Study 9 peptides**
        i. Look at pressure trace to make sure sample (not air) was injected onto column.
        ii. Open up the last SSS acquired before file with missing peaks
            1. In Skyline or in vendor specific software
        iii. Are 22 peaks present?
            1. Yes: go on to point iv.
            2. No: identify which peaks are missing
                a. Check pressure trace to make sure air was not injected onto column and that column pressure isn't too high
                b. Is there enough sample in all vials (sample vials and reagent vials)?
        iv. Are peak shapes symmetric and well defined as opposed to mis-shapen and jagged?
            1. Yes: go on to point v.
            2. No: check ESI tip of column, clean if necessary.
                a. Also check pressure trace to make sure pressure is not too high.
        v. Is column pressure too high?
            1. Compare column pressure of bad runs to previous runs in the sample list
                a. If pressure is too high, remove column and clean tip
                    i. Wipe tip with gloved hand, wet with water and wipe again. Check tip under microscope if possible.
                    ii. If this does not work, check for blockages in transfer lines closest to column and work backwards to LC.
                    iii. If this does not work, consider replacing column.

vi. Is column pressure too low?
    1. If pressure is too low, check all connections between column and LC.
vii. Are flow rates calibrated correctly? (potentially check or recalibrate flow rates)
viii. Are samples being picked up properly from the autosampler vials?

b. Symptom: Retention Time Drift

i. Observe RT shift of the SSS samples in a single Skyline document
    1. Does RT shift in one direction or randomly?
ii. If in one direction, are the peaks migrating out of the 2 min RT window for the Study 9 method? Solution: Re-scheduling because of shifting retention times
    1. Re-run Study 9-1_A with the "heavy only" scheduled method that has a 4 minute RT window.
    2. Import data into an empty Skyline template for Study 9 peptides and export a new scheduled method based on the RT data for light and heavy peptides (738 transitions).
    3. Keep close eye on RT shifts.
    4. Consider running SSS runs at points when you would not be able to check the RT shift and if peaks are in danger of shifting out of RT window (like overnight).

c. **If any concentration points must be re-run, it is important to follow this procedure**:

i. Run SSS sample to ensure system is working properly. Look for smooth, symmetrical peaks with stable retention times.
ii. Inject Sample Study9-1_A before re-running a calibration point to make sure there is little carryover.
iii. Follow with another SSS run so that you have time to analyze the data before running the next concentration point.

HPLC Chromatography Conditions for Studies 9-1:

Individual CPTAC sites are expected to implement these HPLC conditions for the duration of the study. Prepacked New Objective HPLC Columns are provided.

- HPLC-plumbing: DIRECT INJECTION
- Sample Loop: 1 µL sample loop
- Columns for AB and Thermo HPLC systems: (New Objective custom-packed as previously shipped to all sites): PicoFrit 75 µm ID / 10 µm tip (ReproSil-Pur C18-AQ, 3µm, 120Å, length of 12 cm)
- Columns for Waters UPLC systems: a Waters specific column will be used (1.7µm BEH130 C18, 75um X 150mm column)
- Columns for Agilent ChipCube systems: NanoChip
- Mobile phases: (A) 0.1% Formic acid (v/v); (B) 90% Acetonitrile / 0.1% Formic acid (v/v)
- Flow rate: 300 nL/min

- Injection volume: 1 µL on column (full loop injection with overloading the loop with 2 µL)
- Loop for Eksigent LC: 1 µL PEEKsil loop provided in sample kit, 100 µm ID PEEKsil.
- Injection Amount: ~0.5 µg total protein on-column
- Gradient: for details see below, **NOTE: Study 9S system suitability has a slightly different gradient than the new Study 9-1 gradient. Please use the appropriate gradient for these different applications.**

Table 1: Eksigent/Tempo Notes: Autosampler set-up, direct injection mode (representative example)

Autosampler Program with Standard Injection*

#	Function	Command		
1	Output	1-Off		
2	Output	2-Off		
3	Valve	Injector Load		
4**	Aspirate	10 µL Reagent-1	Speed:1	Height:5
5	Aspirate	2 µL Sample	Speed:1	Height:2
6**	Aspirate	2.3 µL Reagent-1	Speed:1	Height:5
7	Output	2-On		
8	Valve	Injector Inject		
9	Dispense	14.3 µL Waste	Speed:5	Height:0
10 **	Needle Wash	200 µL		
11	End			

NOTE: Different gradients are used for Study 9-1 calibration curve (Study 9-1 gradient) vs. system suitability samples (Study 9S gradient) vs. Wash gradient used in between blinded samples (Study 9 Wash gradient).

Table 2a. 9-1 HPLC gradient for all analyses of 123 target synthetic peptides.

Time (min)	%A	%B	Flow Rate (nl/min)
0	97	3	300
5	97	3	300
8	93	7	300
35	75	25	300
42	60	40	300
45	10	90	300
49	10	90	300
50	97	3	300
80	97	3	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 90% acetonitrile/0.1% formic acid (v/v) in water

Table 2b. 9S HPLC gradient to be used for System Suitability Sample (Michrom 6 protein mix).

Time (min)	%A	%B	Flow Rate (nl/min)
0	97	3	300
5	97	3	300
8	85	15	300
42	65	35	300
45	10	90	300
49	10	90	300
50	97	3	300
80	97	3	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 90% acetonitrile/0.1% formic acid (v/v) in water

Table 2c. 9-Wash HPLC gradient to be used for washing the column in between Blinded Samples.

Time (min)	%A	%B	Flow Rate (nl/min)
0	97	3	300
15	10	90	300
16	97	3	300
31	10	90	300
32	97	3	300
62	97	3	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 90% acetonitrile/0.1% formic acid (v/v) in water

NOTE: Total peptide amount greater than 1 µg injected onto nanoLC columns can result in poor chromatographic peak shape and poor reproducibility from run to run. The MARS-14 depleted plasma samples (Samples 9-1-A through 9-1-J, and 9-1-Blank) have been diluted such that a 1 µL injection results in approximately 0.5 µg of total protein on-column. Therefore, they should be analyzed without any additional dilution.

For Waters operators only:

Operators with Waters Aquity UPLC will use a slighly adjusted gradient, due to the use of Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC.

Adjusted Table 3a., for Waters UPLC only. Waters 9-1 HPLC gradient for all analyses of 123 target synthetic peptides.

Time (min)	%A	%B	Flow Rate (nl/min)
0	97.3	2.7	300
5	97.3	2.7	300
8	93.7	6.3	300
35	77.5	22.5	300
42	64	36	300
45	19	81	300
49	19	81	300
50	97.3	2.7	300
80	97.3	2.7	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 100% acetonitrile/0.1% formic acid (v/v) in water

Adjusted Table 3b., for Waters UPLC only. Waters 9S HPLC gradient to be used for System Suitability Sample (Michrom 6 protein mix).

Time (min)	%A	%B	Flow Rate (nl/min)
0	97.3	2.7	300
5	97.3	2.7	300
8	86.5	13.5	300
42	68.5	31.5	300
45	19	81	300
49	19	81	300
50	97.3	2.7	300
80	97.3	2.7	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 100% acetonitrile/0.1% formic acid (v/v) in water

Adjusted Table 3c. for Waters UPLC only. Waters 9-Wash HPLC gradient to be used for washing the column in between Blinded Samples.

Time (min)	%A	%B	Flow Rate (nl/min)
0	97.3	2.7	300
15	19	81	300
16	97.3	2.7	300
31	19	81	300
32	97.3	2.7	300
62	97.3	2.7	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 100% acetonitrile/0.1% formic acid (v/v) in water

Document S2: Study 9.2 (Phase III) SOP

Study 9-2 SOP (Phase III) for NCI CPTAC Consortium-Wide Multiple Reaction Monitoring (MRM) Experiment

9-Point Calibration Curve of 27 ^{15}N labeled target proteins monitoring 125 target peptides in 3 different isotopically labeled forms

Experimental Design and Statistics Verification Studies Working Group

Overview

In Study 9.2, there will be 27 undigested ANL target proteins and 7 undigested "Study 7" proteins that will be centrally spiked into depleted human K_2EDTA plasma. Preparation of a standard curve will be performed at Vanderbilt prior to distribution of the sample kits. A detailed SOP for digestion, subsequent sample handling, and instrumental analysis will be included for each lab (also see Appendix A for chemical reagents needed). For Study 9-2, the 9-point calibration curve will span from 10 amol/µL to 100 fmol/µL in a background of MARS-14 depleted human K_2EDTA plasma (500 ng/µL). The assay will monitor for three isotopic forms of 115 peptides from 27 cancer-relevant proteins (light, $^{13}C/^{15}N$, and uniformly labeled ^{15}N, the latter from here on referred to as U-^{15}N), as well as two isotopic forms (light and $^{13}C/^{15}N$) of 10 control peptides by time-scheduled LC-MRM-MS, for a total of 1095 transitions. Each participating CPTAC site must strictly follow the specifications of the SOP as outlined. Results from Study 9.2 will mimic a "real world" verification study in which each site is responsible for sample preparation. Study 9.2 also rigorously tests the transferability and reproducibility of MRM-MS-based assays for target proteins in plasma across multiple institutions and across 4 major MS instrument vendors and multiple platforms.

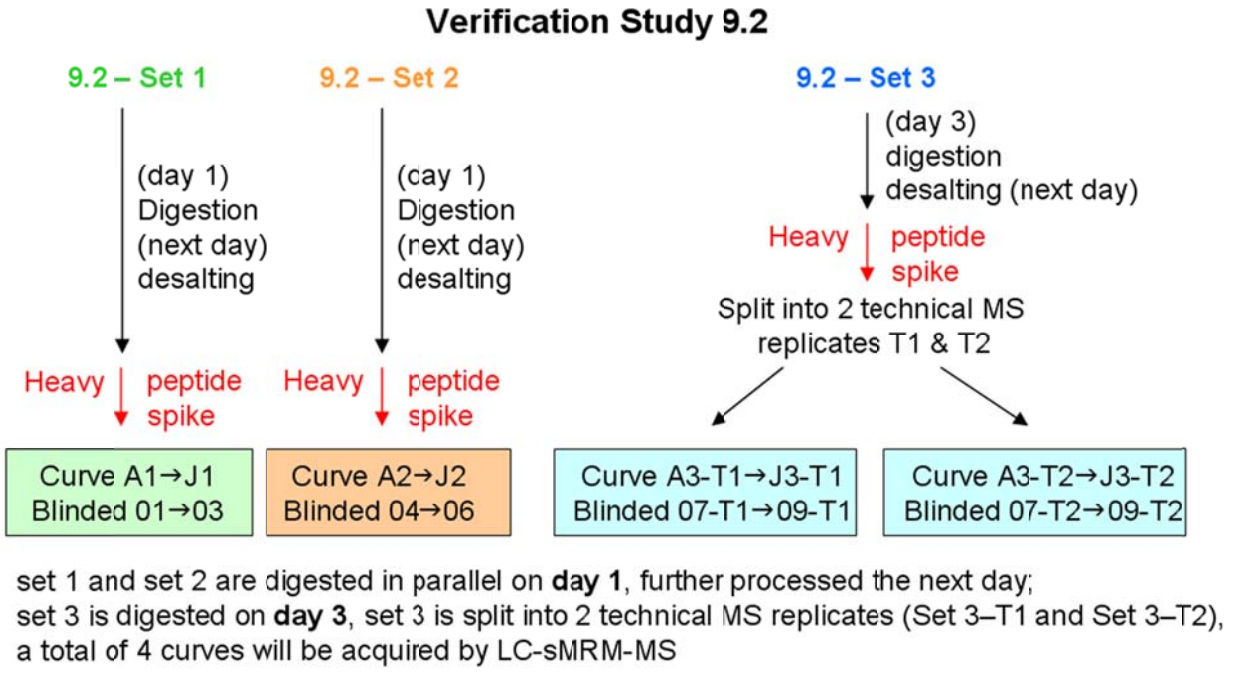
Overall, 27 U-^{15}N -labeled proteins will be spiked into depleted plasma at a constant spike level, which after digestion and dilution (that will be performed at each CPTAC site) will give rise to 115 target peptides at a concentration of 25 fmol equivalent on column. Twenty-seven light proteins will be spiked in at varying levels with final concentrations after digestion/dilution ranging from 10 amol to 100 fmol on column. Seven light "Study 7" proteins will be spiked in at constant levels providing a digestion reproducibility control, and after digestion and dilution yield final concentrations of 2.5 fmol equivalent on column. One hundred twenty-five synthetic $^{13}C/^{15}N$ signature peptides will be spiked in by each operator after digestion and desalting at concentrations that will yield final concentrations of 10 fmol on column.
As an additional phase of the study, three blinded samples will be included with each curve replicate, in which the spiked concentration of the light target proteins is unknown to the operators. As with the curve samples, each site will be responsible for the sample preparation of the blinded samples.

Unlike previous studies, we will ship 3 sets of samples (Set1, Set2 and Set3) with undigested proteins spiked into undigested depleted plasma (each set consists of a curve and blinded samples).
Even though only 3 sets of samples will be shipped, there will be 4 singlicate curves to be acquired. Set 3 will be split in half after processing as described in detail below and it will yield technical replicates Set3T1 and Set3T2 (also see Scheme 1). This will reduce digestion efforts for operators. The digestions (similar to study 7.3) will be performed at each participating

CPTAC site. Digestion replicates (sets) 1 and 2 will be digested in parallel at the same time on "day 1", and then analyzed on the triple quadrupole instruments, as singlicate curves and blinded samples 1 and 2 (digestion replicates one and two). The sample set 3 should be digested shortly after sample sets 1 and 2, preferably on "day 3" (for logistical reasons it can also be later than "day 3", it just should be done on a separate day). For details on how to split Set 3 after processing: Set 3 will be split in half after completion of the following steps i) digestion, ii) desalting, and iii) heavy $^{13}C^{15}N$ peptide spike, and will be acquired as technical replicates Set3T1 and Set3T2. Set3T1 and Set3T2 will be frozen separately and acquired later independently as curves 3 and 4, respectively.

The schematic illustrating the workflow for each 9.2 sample set is shown below in Scheme 1. Please note: Sets 1 and 2 should be digested on the same day, while Set 3 should be digested on a later day.

Scheme 1:



For all sample sets, after digestion, desalting, and heavy peptide spike operators will be asked to aliquot the solutions (for details see below).

It would be ideal if, at each site the same person could do all digestions for an individual instrument platform in order to keep variability to a minimum (otherwise, please keep track of personnel doing the different digestion steps).

Aims: The study described in this SOP is designed to accomplish the following:

1. To generate a 9-point standard curve for 27 cancer-relevant protein targets spanning 10 amol/µL to 100 fmol/µL in a depleted plasma background (500 ng/µL) that will be analyzed by LC-MRM-MS on four different triple quadrupole platforms at CPTAC sites.

2. To enable calculations of LOD, LOQ, accuracy and precision from the calibration curve for all 115 peptides that will be monitored after protein digestion performed at each site.

3. To monitor digestion reproducibility and efficiency across all samples by addition of seven "Study 7" proteins at constant levels in all samples.

4. To introduce a set of 3 blinded samples that mimic real biomarker samples into the sample handling workflow and assess the quantitative reproducibility across all sites.

5. To evaluate the level of endogenous and interfering signals for all peptides by replicate measurements of blank plasma samples. As in this study 115 different peptides are monitored the aspect of endogenous and interfering signals can be assessed more comprehensively than comparison to Study 7.

6. To observed the improvement in protein-level quantification through use of U-15N proteins as internal standards.

The number of transitions for 3 different label types that will be targeted in this method (1095) is a critical challenge and will require the use of retention time scheduled LC-MRM-MS. To achieve success, the following protocol is designed to assess the system suitability and retention time stability of the LC-MRM-MS instrument, as well as design a single MRM-MS method empirically. The protocol outlined herein should be followed as strictly as possible and all deviations from this protocol must be outlined in detail on the last sheet. The experimental design is outlined below.

Experimental Outline

1. Digest samples according to the digestion protocol.
2. Run System Suitability Sample 5 times to condition column and assess LC-MRM-MS performance. Only proceed to the following steps if the system suitability samples pass defined criteria.
3. Using a Skyline template for heavy target peptides (download from NIST FTP server), create 6 MRM-MS transition lists for the heavy target peptides only (maximum of 63 transitions in each unscheduled method)
4. Analyze the heavy-only peptide sample using the 6 different MRM-MS methods
   i. Import the 6 raw files into Skyline
   ii. Obtain retention times for each heavy target peptide
   iii. Export a scheduled MRM-MS transition list (or MRM-MS method) with a 4 min RT window

iv. Run the scheduled LC-MRM-MS method twice using one processed sample vial 9.2-A that will be digested at each CPTAC site.
v. Import the 2 raw files into Skyline
vi. Download a second Skyline template from the NIST server containing **heavy ($^{13}C/^{15}N$), light (unlabeled) and U-^{15}N -labeled peptides** (download the new template rather than adding in new label types into the existing heavy only template!).
vii. Export a new scheduled LC-MRM-MS method with all 1095 transitions and a 2 min RT window. For instruments that allow for flexible RT window scheduling (i.e. Waters Xevo QQQ, Agilent 6400 series and Thermo Vantage instruments), adjustments can be made deviating from the 2 min window for selected problematic peptides. Please make sure the number of concurrent transitions does not exceed 100.
viii. Run sample 9.2-A with the 2 min RT window method prior to running the cal curve.

5. Execute calibration curve and blinded samples according to specified run order.
6. Import data into Skyline and integrate.
   i. Import System Suitability Sample files into System Suitability Skyline file and integrate. Ensure peak area CVs and retention times are within specified error limits.
   ii. Import Calibration Curve files into Study 9-2 Curve Skyline file and integrate.
   iii. Import Blinded Sample files into Study 9-2 Blinded Sample Skyline file and integrate.
7. Export reports from each Skyline file (note: a new, different Study 9.2 Skyline report template will be provided) and process data using QuaSAR to monitor for data quality. Make any adjustments necessary to data integration and re-run samples as necessary.
8. Upload the 3 generated Skyline reports to the NIST FTP server along with the 3 different Skyline files and all raw MRM-MS data files (see separate document).

Materials and Reagents Provided in Kit

A. Study 9-2-SSS (System Suitability Sample)
- a. Digest of 6 equimolar proteins (Michrom Bioresources, #PTD/00001/63)
  - i. Four 10 µL aliquots, 1 pmol/µL, supplied in 30% acetonitrile/0.1 % formic acid in water

B. Heavy-Only IS Peptide Mixture for Scheduling only (in 25 fmol/uL 6-protein mix)
- a. 125 heavy IS peptides at **800 fmol/µL each**
  - i. Two 10 µL aliquots, supplied in 30% acetonitrile/0.1% formic acid

C. QC Sample (Sample 9-2-QC)
- a. Equimolar mixture of the 125 unlabeled and 125 labeled synthetic peptides in 25 fmol/µL 6-protein mix matrix
  - i. Four 25 µL aliquots supplied at 10 fmol/µL of each peptide in 0.1% formic acid in water. Background of 25 fmol/µL of 6-protein mix added to minimize adsorption/loss of hydrophobic peptides.

D. Sample 9.2-A that will be digested at each CPTAC site (on-site digestion), for scheduling and as "blank in between blinded samples" use
- a. Each 9.2-A vial contains depleted plasma at ~4.5 µg/µL spiked with 27 U-^{15}N-labeled proteins and 7 light digestion control proteins. Depleted digested plasma will be diluted to a final concentration after digestion of approximately 0.5 µg/µL
  - i. **Four 9.2-A vials, 35 µL each; vials A should be digested along with the other study samples. Referring to Scheme 1, two A vials can be digested on day 1 (along with sample sets 1 and 2), while the other two A vials can be digested on day 3 (along with sample set 3).**
  - ii. After desalting the sample will be spiked with 125 heavy ^{13}C/^{15}N IS peptides (stock at 500 fmol/µl, see Reagent F).
  - **iii.** After dilution, the sample will ultimately contain 125 ^{13}C/^{15}N-labeled peptides at 10 fmol/µL, 115 U-^{15}N-labeled peptides at 25 fmol/ul equivalent in 500 ng/µL digested depleted plasma (no light ANL proteins were spiked).

E. Samples 9-2-B to 9-2-J: Non-digested human depleted plasma spiked with 27 light and 27 U-^{15}N-labeled ANL proteins, and 10 light digestion control proteins
- a. Depleted plasma, 500 ng/µL
  - i. Three sets of 35 µL aliquots of each spike level are supplied (of which 25 µL from each vial will be digested on site). Set 3 will be split into 2 technical replicates T1 and T2 after digestion/desalting to yield a final number of 4 singlicate curves (see Scheme 1 above).
  - ii. 27 U-^{15}N-labeled proteins (yielding 115 target peptides after digestion) spiked at a concentration of 25 fmol/µL on column.
  - iii. 27 unlabeled proteins (yielding 115 target peptides after digestion) were spiked in at the following concentrations (note, these concentrations are different from the 9.1 concentration points). *Concentrations in the table below refer to shipped light target protein concentrations, and then after digestion resulting final on-column peptide equivalent concentrations, assuming theoretical 100% recovery).*

Sample (Study 9-2)	Spiked light target Protein Concentration (fmol/µL)	On-column Peptide Equivalent Concentration (fmol/µL)
9-2-J	900	100
9-2-I	213	24
9-2-H	51	5.6
9-2-G	12	1.3
9-2-F	7.4	0.82
9-2-E	2.8	0.32
9-2-D	0.68	0.075
9-2-C	0.16	0.018
9-2-B	0.09	0.010

Note: the above protein/depleted plasma concentration points also contain 7 undigested light "Study 7" proteins spiked at constant concentration yielding a final of 2.5 fmol equivalent on column, assuming 100% recovery.
After digestion/desalting and spiking of the heavy synthetic $^{13}C^{15}N$-labeled IS peptides (see reagent F), each of the resulting 4 different singlicate calibration curves can be acquired: Specific naming including the first blank (A sample) is required, for details also see run order below:
9-2-A1 to 9-2-J1; 9-2-A2 to 9-2-J2;
9-2-A3-T1 to 9-2-J3-T1; 9-2-A3-T2 to 9-2-J3-T2.

F. Heavy IS Peptide for Spike after Digestion, 500 fmol/µL

- **a.** One aliquot of 200 µl aliquot of 125 heavy synthetic $^{13}C^{15}N$-labeled IS peptides
- **b.** Supplied at **500 fmol/µL** in 30% acetonitrile/0.1% formic acid
- **c.** 4.5 µl need to be added (at this undiluted concentration 500 fmol/ul) by the operator to all samples after digestion and desalting to yield final concentrations of 10 fmol on column.

G. Blinded Samples

- **a. Nine blinded samples, 35 µL in depleted plasma,** (Total: Samples 9-2-01Blinded to 9-2-09Blinded)
- b. To be digested and to be run after each singlicate calibration curve
- **c.** Three sets of three Blinded samples (each provided in 35 µl aliquot, of which 25 µl are digested), with Set 3 being split into two technical replicates T1 and T2 after digestion and desalting (refer to Scheme 1):
  - **i.** first singlicate: 9-2-01Blinded to 9-2-03Blinded
  - **ii.** second singlicate: 9-2-04Blinded to 9-2-06Blinded
  - **iii.** third singlicate: 9-2-07-T1Blinded to 9-2-09-T1Blinded
  - **iv.** fourth singlicate: 9-2-07-T2Blinded to 9-2-09-T2Blinded
- **d.** For each singlicate: the three blinded samples will be run twice as runs a) and b), see runorder.

H. Wash Samples

- **a.** To be prepared from the 6-protein mix / Michrom sample at stock concentration of 1 pmol/µL (Reagent A, Study 9-2 SSS vial, see above).

b. **Wash Samples** will be 10 fmol/µL of the 6-protein mix (Michrom sample). Samples will be diluted from provided stock concentration of 1 pmol/µL).
c. Will be used to avoid carryover between blinded samples, the run order defines running a "wash" gradient in between blinded samples. "

I. Chemical Reagents for Sample Preparation, Digestion and Buffer Solutions for digestion are detailed in Appendix A. Several but not all of these reagents are included in the sample kit, please review which additional reagents your laboratory may need to obtain (Appendix A)

J. HPLC solvents
a. Acetonitrile, HPLC grade
b. Water, HPLC grade
c. Formic Acid

K. HPLC columns
a. General specifications: C18 packing material in column dimensions of 75 µm ID x 12 cm with 10 µm ID tip
b. AB SCIEX and Thermo operators: Prepacked New Objective Reprosil Columns may be requested from New Objective. Limited to a total of 5 per site, including those used in Study 9.1. If more than 5 columns are required, the cost for additional columns will be the responsibility of the site.
c. Waters UPLC operators: a Waters specific column will be used (1.7µm BEH130 C18, 75 µm X 150 mm column)
d. Agilent 6400 series operators with ChipCube sources, please use Chips packed with Zorbax C18 (5 um and 300 angstrom beads) with 160 nL enrichment column and 75 µm ID x 15 cm analytical column.

L. Autosampler loop for HPLC systems: 1 µL PEEKsil loop provided in sample kit, 100 µm ID PEEKsil; newer design 1 µL loops are provided for Eksigent Ultra systems.

M. Fused silica
a. 25 µm ID between gradient pump and autosampler (for direct inject configuration)
b. 25 µm ID between autosampler and PicoFrit column (keep tubing as short as possible)

N. High voltage contact
a. For liquid-liquid junction, Upchurch part P-888.

O. Autosampler vials
a. Polypropylene autosampler vials with conical insert, 250 µL maximum volume

P. Column heater
a. If the system is equipped with a column heater, please set to 35 degrees Celsius.

Q. **Oasis HLB desalt cartridge** (Waters)
a. Off-line desalting will use Waters Oasis HLB 1 cc, 30 mg cartridges (Product # WAT094225, box of 100).
b. A vacuum manifold (Product # WAT200677) and vacuum source will be required for the cartridges. Each participating lab is responsible for ordering the necessary equipment. *For operators who are experienced with Oasis elution plates those can be used as well. The product number for the Waters Oasis HLB uElution Plate (30 um) is 186001828BA (Qty/Box=1) with corresponding extraction manifold with product number 186001831. (however as these plates are not as trivial to use we rather recommend to use the individual cartridges, see above).*

Required Software

A. **Skyline Daily Software**, most recent version (please note version used)
B. **Platform-specific Skyline document** (*.sky), provided by CPTAC VWG on NIST ftp site (**ftp://chemdata.nist.gov/verification_main_study9.2/Skyline_templates/**)
C. **QuaSAR Quantatitive Statistical Analysis for Reaction Monitoring Experiments** (available at GenePattern test site: http://genepatterntest.broadinstitute.org/gp/pages/login.jsf)

Overview

In Study 9-2 (9-point calibration curve), 27 U-^{15}N-labeled proteins will be spiked into depleted human K_2EDTA plasma at a constant spike level, which after digestion, desalting, and dilution (performed at each CPTAC site) will result in 115 U-^{15}N-labeled target peptide equivalents at a concentration of 25 fmol on column (assuming 100% digestion efficiency and recovery). The U-^{15}N-labeled proteins will serve as internal standards to provide more accurate protein quantification. Twenty-seven light proteins will be spiked in at varying levels with final concentrations after digestion/dilution ranging from 10 amol to 100 fmol on-column. Seven light "Study 7" proteins will be spiked in at constant levels providing a digestion reproducibility control, and after digestion and dilution will yield final concentrations of 2.5 fmol on column (assuming 100% recovery). One hundred twenty-five synthetic ^{13}C/^{15}N-labeled signature peptides will be spiked in by each operator after digestion and desalting to yield final concentrations of 10 fmol on column. Their use is to assess digestion recovery.
Digestion of each prepared concentration point and blinded samples will be performed at individual CPTAC sites. Results from Study 9-2 from all CPTAC sites will provide the following metrics:

- LOD/LOQ values for the 115 peptide targets in depleted plasma based on ^{13}C/^{15}N synthetic internal standard peptides
- Overall recovery of each peptide from the sample handling process, based on ^{13}C/^{15}N synthetic internal standard peptides
- Improvements in quantitation precision by the use of U-^{15}N-labeled proteins as internal standards
- Digestion reproducibility across assays (intralaboratory) and across sites (interlaboratory), based on the 7 proteins used as digestion standards
- Overall reproducibility both intralaboratory and interlaboratory for the quantification of blinded samples with unknown concentrations

The following paragraphs provide a description of the experimental procedure, with each major step separated into its own paragraph and heading. Paragraphs are followed by outlined "checklists" that have matching headings for reference.

1. Sample Preparation: Denaturation, Reduction, Alkylation, Digestion, Desalt

All protein samples (calibration curve points A-J, and blinded samples) are provided in a

matrix of undigested depleted plasma. All samples will have to be denatured with urea, reduced with DTT, alkylated with iodoacetamide, and diluted prior to addition of proteolytic enzymes. Stock solutions of Lys-C and Trypsin will be provided to allow a double digestion protocol. It is recommended to begin the denaturation process early in the day (at least by noon) so that the reduction, alkylation, and addition of Lys-C with 2 hour incubation can be completed before the end of the day. Final addition of Trypsin should occur approximately 16 hours before you are able to quench the reaction. Each sample should be quenched with formic acid so that the final concentration in solution is 1%, then subjected to desalting on an Oasis HLB cartridge (Waters). The $^{13}C/^{15}N$ internal standard peptide mix will be added after desalting and lyophylization, immediately prior to analysis by LC-MRM-MS. After addition of the IS peptides, sample aliquotting should be performed as described below. Curve replicates 1 and 2 (along with associated blinded samples) should be digested at the same time, while curve replicate 3 should be reserved for preparation on a separate day (to assess day-to-day variability in sample handling). Replicate 3 should also be split in half after reconstitution with the $^{13}C/^{15}N$ internal peptide standards and will be analyzed twice to assess technical variability in the assay (generating curves 3 and 4).

2. System Suitability Sample, Column Conditioning and Instrument Performance

System Suitability Sample (SSS) runs using the Michrom 6-protein mix (based on Study 9S) will be periodically interspersed into Study 9-2 to guarantee system suitability and performance and, in particular, to monitor peak area stability and RT drift. The SSS will first be analyzed in an unscheduled LC-MRM-MS method (Study 9S) to condition the column and assess the performance of the LC-MS instrument platform prior to initiation of the quantitative assay. Upon obtaining the specified results for retention time and peak area (**RT drift should be <1min; peak area CV should be <20%, certainly <30%**), sites may proceed to the generation of the scheduled LC-MRM-MS runs for Study 9-2.

The SSS will also be run every 6-8 runs in the sample queue, in order to track instrument performance. Data will be analyzed in Skyline and will be submitted in a separate "Michrom Study 9S" file.

3. Generation of Scheduled LC-MRM-MS Method for Study 9 Peptides

In preparation for the scheduled calibration curve LC-MRM-MS runs, in which 1095 transitions will be monitored in one run, participating sites will monitor all heavy synthetic labeled peptides with 375 transitions (125 peptides with 3 transitions each) in six unscheduled LC-MRM-MS runs (~63 transitions per run), with each run represented by a separate LC-MRM-MS method. Retention times for scheduling will be determined empirically using these six runs and will be verified by analysis with a single scheduled LC-MRM-MS method (acquired twice) prior to analyzing the samples from which the calibration curve will be generated. All method building and data analysis will be performed using Skyline (please make sure to have downloaded the most recent Skyline daily version as of your data acquisition date).

4. Data Analysis

Operators will import all acquired data files into Skyline to check and, if necessary, adjust peak integration parameters. Check "Integrate All" (Menu, Settings, Integrate all), so that light, heavy

and U-15N transitions will be integrated together. While checking peak integration open RT replicate views (Menu, View, Retention Time, Replicate Comparison), and Peak Area replicate views (Menu, View, Peak Areas, Replicate Comparison) to visually help confirm proper peak integration. In Study 9-2, there will be three forms of each peptide monitored, and all should co-elute. The $^{13}C/^{15}N$ and U-^{15}N-labeled forms of the peptides should be present at consistent levels across all samples. Check for RT drift problems during scheduling, and ensure that the correct peak has been integrated (helpful hint: look for co-elution of the three forms of each peptide, and specifically, the $^{13}C/^{15}N$ and U-^{15}N-labeled forms should always be coeluting). It is recommended to not "fine tune" the automatic Skyline peak integration, unless it is obvious that the wrong peak has been integrated (more likely in the lower concentration samples). Use Skyline Custom Annotation features to annotate any observations or notes for Study Statisticians to consider (Menu, View, Results Grid; and annotate observations on the precursor or transition level). Finally use "QuaSAR" to analyze data before submitting data to statisticians (see separate SOP). The experimentally determined molar concentration of the spiked peptide or protein will be calculated and compared to its theoretical value for accuracy. QuaSAR will generate linear plots of response versus known concentration from each of the 9-point standard curves and will be used to evaluate the linearity of the MRM measurements across the range of spiked peptide concentrations, thus providing evidence of a quantitative measurement process. Samples with high %CV (>20%) and/or interferences will be flagged on the plots. Replicate analyses of the spiked plasma samples will provide estimates of assay precision (standard deviation and % CV), and LOQ and LOD will be determined. Blank runs of digested plasma with labeled peptides and ^{15}N ANL proteins (referred to as sample 9.2-A) will provide estimates of chemical background levels in the absence of unlabeled signature peptide peaks as well as the presence of endogenous peptides in the sample (*i.e.*, CRP). Furthermore, an estimate of carryover will be determined by running a series of gradient HPLC washout runs. Finally, variation across CPTAC sites will be assessed for each of these characteristic analytical metrics.

5. Troubleshooting

This study contains a large number of samples that need to be run in a defined order and requires very reproducible peptide RTs. Problems may arise that will affect RT stability, including, but not limited to, increased column pressure, tip blockage, significant change in ambient temperature or injection of air into the system. The troubleshooting section provides some suggested routes of diagnosis and an outline of how to resolve problems and continue with data acquisition. Importantly, operators are instructed to use the SSS and Skyline to observe any problems with retention time shifting, asymmetric peaks, and low signal. These problems can be caught and rectified without much down time and with minimal re-running of sample.

Procedures

1. Step by Step Sample Preparation/Digestion Procedures:

Note: at each of the steps perform appropriate vortex and spin-down steps (particularly to assemble the sample at the bottom of the tube after incubations). Under appendix A point II, page 37, you can find further details on procedures for Digestion Reagent Preparation (please review before starting, specifically how to prepare digestion reagent B, 9M urea in 100 mM Tris).

A. Digestion Protocol for Plasma Samples.

A. Each undigested protein/depleted plasma sample vial will contain a volume of 35 µL. Let the sample thaw at room temperature. Centrifuge briefly in a benchtop centrifuge and remove 25 µL out of each vial (that originally contained 35 µL) and transfer to a new tube.
B. To those 25 µL of the undigested protein/depleted plasma mixture, add 50 µL of 9M Urea in 100 mM Tris pH 8 (digestion reagent B, Appendix A). The final concentration of Urea will be 6M. [Approximate protein concentration prior to dilution is ~4.5 µg/µL].
C. Add 8.3 µL of 200 mM DTT [final concentration of 20 mM]; incubate for 30 min at 37°C. (refer to digestion reagent D, Appendix A)
D. Add 9.3 µL of 400 mM M IAM [final concentration of 40 mM]; alkylate at room temperature for 30 min in the dark. (refer to digestion reagent E, Appendix A)
E. Add 135 µL of 100 mM Tris, pH 8 (refer to digestion reagent C, Appendix A) to to reduce the urea concentration to 2M.
F. Add 6.4 µL of 0.4 µg/µL LysC solution. (about 135 ug total protein to digest)
G. Check the pH of the digestion solution with pH strips and if needed adjust it to 8.0-8.5 with 1M Tris solution, pH 8 (refer to digestion reagent A, appendix A). If you do need to adjust, please carefully add 1-2 µL 1M Tris, pH 8 at a time (digestion reagent A) so the solution does not get too basic).
H. Incubate at 30°C for 2 hours with shaking at 850 rpm.
I. After 2 hours add 600 µL of 100 mM Tris pH 8 to each tube to reduce the urea concentration to < 1 M.
J. Dissolve one trypsin vial (100 ug per vial) of Promega Trypsin Gold in 1000 uL of 100 mM Tris, pH 8.0 (digestion reagent C, Appendix A). Keep trypsin solution on ice and use quickly after preparation to avoid autolysis. Add 25.5 µL of this freshly prepared 0.1 µg/µL trypsin solution to each digest sample with gentle mixing. Use one fresh tryspin vial for digestion of sample sets 1 and 2 on day 1 and a second fresh trypsin vial for digestion set 3 (on a separate day, i.e. day 3).
K. Check the pH of digest solution with pH strips and if needed adjust it to 8.0 with 1M Tris solution, pH 8 (refer to digestion reagent A, appendix A). If you do need to adjust, please carefully add 1-2 µL 1M Tris at a time (digestion reagent A) so the solution does not get too basic).
L. Incubate overnight (16 h) at 37 °C with shaking at 850 rpm.
M. Add 9 µL concentrated formic acid to each digest to quench enzyme activity for a final acid concentration of 1%.

NOTE the difference between Reagent B (Heavy-Only IS Peptide Mixture for Scheduling only with 125 heavy IS peptides at 800 fmol/µL) each, and Reagent F (Heavy IS Peptide for Spike after Digestion: 125 heavy synthetic $^{13}C^{15}N$-labeled IS peptides, supplied at 500 fmol/µL). While Reagent B is initially used for RT scheduling (after dilution to 80 fmol/µl), Reagent F will be used at the given concentration (500 fmol/µL) to spike in 4.5 µl for each sample after digestion, desalting, reconstitution.

B. Offline Desalting of Digest Solutions via Oasis HLB SPE Cartridges

i. Each digest requires off-line desalting using Waters Oasis HLB 1 cc, 30 mg cartridges (Product # WAT094225, box of 100). A vacuum manifold (Product # WAT200677) and vacuum source will be required for the cartridges. Each participating lab is responsible for ordering the necessary equipment. Part numbers were provided previously to help each lab procure these items.

ii. Condition cartridge with 3 x 400 µL of 0.1 % formic acid in 80 % ACN.

iii. Equilibrate cartridge with 4 x 400 µL of 0.1 % formic acid in 100 % water.

iv. Reduce flow rate by lowering vacuum. A slower flow rate during sample loading, washing and eluting will minimize sample loss and maximize salt removal.

v. Add sample to cartridge.

vi. Wash cartridge with 4 x 400 µL of 0.1 % formic acid in 100 % water.

vii. Elute plasma digest peptides with 3 x 400 µL 0.1 % formic acid in 80 % acetonitrile into 1.7 mL Eppendorf tubes.

viii. Freeze eluates on dry ice or at -80 °C for approximately 1 hour. Lyophilize to dryness. Samples can be stored lyophilized at -80 °C until ready for MRM-MS analysis.

C. Sample Reconstitution and $^{13}C/^{15}N$ IS Peptide Spikes, Reagent F (500 fmol/µL), to be performed just prior to executing LC-MRM/MS

i. Reconstitute dried and desalted plasma digests with 25 µL of 5% formic acid, 3% acetonitrile, 92% water and vortex. To make this solution prepare 1 ml aliquots as needed (v/v/v: start with 920 ul water, then carefully add 50 ul formic acid, and finally add 30 ul acetonitrile).

ii. Add 4.5 µL of 500 fmol/µL $^{13}C/^{15}N$ IS peptide mixture (see Reagent F above) to each of the digestion solutions.

iii. Add 195.5 µL of water to achieve final $^{13}C/^{15}N$ peptide concentration of 10 fmol/µL and the plasma digest concentration is 0.5 µg/µL.

iv. This will yield a total volume of 225 µL per each sample after digestion, desalting, reconstitution, $^{13}C/^{15}N$ IS peptide spike, and final dilution.

v. **Aliquot** the final dilution volume into **50 µL aliquots** and freeze aliquots that are not immediately subjected to LC-MRM/MS in the -80 °C freezer.

vi. **Aliquoting of Reagent F:** After Reagent F (with a total volume of 200 µl) is thawed for the first time in order to add reagent F to each of the samples of **Sets 1 and 2**, please generate aliquots of 50 µl volume each to be used for sample **Set 3** to avoid further freeze thaw cycles.

2. <u>System Suitability Sample – Column Conditioning</u>

A. Sample preparation

i. Dilute SSS from 1 pmol/µL to 50 fmol/µL:

ii. Add 95 µL of 0.1% formic acid/3% acetonitrile to an Eppendorf tube

iii. Add 5 µL of MichromMix (SSS) to the vial and vortex

iv. Centrifuge sample for 1 min in a benchtop centrifuge

v. Transfer to an autosampler vial and place in autosampler

B. Method Preparation
   i. Prepare SSS transition list from the appropriate Skyline file
      1. 115 transitions, 22 peptides
      2. Q1, Q3 resolution = unit
      3. Dwell time = 10 msec
      4. Interscan delay (where applicable) = 5 msec (3 msec for QTRAP 5500)
   ii. LC Gradient: use the **Study 9S** LC gradient (also see Table 2b)
      1. 0-5 min, 3% B; 5-8 min, 3-15% B; 8-42 min 15-35% B; 42-45 min, 35-90% B, 45-49 min, 90% B hold; 49-50 min, 90-3% B; 50-80 min, 3% B. *Note: operators with Waters Aquity UPLC will use a slightly adjusted gradient (due to the use of Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC, see Table 3b).*
      2. Flow rate: 300 nL/min
      3. Mobile phase A: 0.1% formic acid in water (v/v); Mobile phase B: 90% acetonitrile (v/v)/0.1% formic acid in water (v/v). *Note: operators with Waters Aquity UPLC will use Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC).*
   iii. Autosampler Settings
      1. Direct Injection, full-loop injection, 1 µL sample loop
      2. Pick up 2 µL of sample sandwiched between mobile phase A (0.1% formic acid)
      3. See Table 1 for example
   ii. Column heater Settings
      1. If using a column heater, please set to 35 degrees Celsius.
   iii. Recommended MS Source Conditions
      1. AB SCIEX (4000 QTRAP and QTRAP 5500)
         a. ESI voltage: 2200 ± 200 V ; Curtain gas: 20 ± 5; GS1: 5 ± 5; IHT: 150 ˚C
      2. ThermoFisher Scientific (Vantage)
         a. ESI voltage: 1300-1400 V; Declustering voltage: -1 V; Capillary Temperature: 210 ˚C
      3. Waters Xevo
         a. Capillary voltage: 2800 V; Cone voltage: 35V; Ion source temperature: 150 ˚C; Cone gas flow: 15 L/Hr; Nanoflow gas flow: 0.20 bar
      4. Agilent 6410/6460/6490 Chip Cube
         a. ESI voltage: 1700-1800 V; gas flow: 2.5 L/min (11 L/min for 6490); temperature: 325˚C (150˚C for 6490); Fragmentor voltage: 125 V (380 for 6490); Cell Accelerator Voltage: 4 V for 6490
   iv. Inject sample 5 times
C. Data Analysis
   i. Import data files into Skyline System Suitability Sample file
   ii. Check automatic integration of all peaks
   iii. Manually adjust integration of peaks, if necessary
   iv. Make sure integration start and stop is identical for all transitions of a precursor (go to “Settings”, and check “Integrate All” to enable this feature automatically)
   v. Document any peak that looks problematic by utilizing the “note” feature in Skyline
      1. Excessive tailing or fronting

2. Drop-out of electrospray
3. Poor peak shape
4. Missing transitions

ii. Check that peaks pass criteria
1. RT shift is isolated to first 3 injections
2. Peak area CV is less than 30% for all peaks
3. All peaks are detected in the last 2 sample runs

iii. If data do not pass criteria, troubleshoot the LC system and re-run SSS column conditioning procedure

iv. If necessary, contact CPTAC VWG members for advice or assistance
1. Susan Abbatiello, susana@broadinstitute.org; 617-714-7653
2. Birgit Schilling, bschilling@buckinstitute.org; 415-209-2079

3. Retention Time Scheduling for Heavy Peptides

A. Sample preparation

i. Dilute Heavy-Only IS peptide mixture from 800 fmol/µL to 80 fmol/µL
1. Add 45 µL 3% acetonitrile/0.1% formic acid to an Eppendorf tube
2. Add 5 µL of the 800 fmol/mL Heavy-Only IS peptide stock to the vial, vortex
3. Transfer to an autosampler vial and place in autosampler

B. Method Preparation

i. Prepare the Heavy-Only IS peptide transition list from the appropriate Skyline file
1. 375 transitions, 125 peptides, 125 precursors
2. Q1, Q3 resolution = unit
3. Dwell time = 10 msec (can be lower for QTRAP 5500 instrument, minimum is 5 msec)
4. Interscan delay (where applicable) = 5 msec (3 msec for QTRAP 5500)
5. Maximum number of transitions per individual unscheduled method: 63
6. Export transition list as multiple methods, ignore proteins

ii. Use the **Study 9-2** LC gradient (also see Table 2a)
1. 0-5 min, 3% B; 5-8 min, 3-7% B; 8-35 min 7-25% B; 35-42 min, 25-40% B, 42-45 min, 40-90% B; 45-49 min, 90% B hold; 49-50 min, 90-3% B; 50-80 min, 3% B. *Note: operators with Waters Aquity UPLC will use a sligthly adjusted gradient (due to the use of Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC, see Table 3a).*
2. Flow rate: 300 nL/min
3. Mobile phase A: 0.1% formic acid in water (v/v); Mobile phase B: 90% acetonitrile (v/v)/0.1% formic acid in water (v/v). *Note: operators with Waters Aquity UPLC will use Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC).*

iii. Autosampler Settings
1. Direct Injection, full-loop injection, 1 µL sample load
2. Pick up 2 µL of sample sandwiched between mobile phase A (0.1% formic acid)
3. See Table 1 for example

iv. Recommended MS Source Conditions (use as guidelines, see above, Section 2.B.iii.)

C. Inject the sample 6 times, one time for each method created in Skyline

i. Name each injection with its own file name/sample name so that each injection is its own, individual file

1. Study9-2_SiteXX_HeavyOnly_method01
2. Study9-2_SiteXX_HeavyOnly_method02
3. Study9-2_SiteXX_HeavyOnly_method03
4. Study9-2_SiteXX_HeavyOnly_method04
5. Study9-2_SiteXX_HeavyOnly_method05
6. Study9-2_SiteXX_HeavyOnly_method06

ii. Follow with 1-5 injections of SSS

1. Do not allow the system to sit idle after the 6 HeavyOnly sample runs

4. Data Analysis

i. Import data files into the appropriate Skyline Platform-specific Study 9-2 file

1. Import multiple files as one replicate
2. Name replicate (HeavyOnly_rep1)

ii. Check automatic integration of all peaks

1. Manually adjust integration of peaks, if necessary
2. Make sure integration start and stop is identical for all transitions of a precursor (check integrate all in Skyline)
3. Document any peak that looks problematic using the "note" feature in Skyline - Use Skyline **Custom Annotation** to note any of the above features as described below (see separate document)
   a. Excessive peak tailing or fronting
   b. Drop-out of electrospray
   c. Poor peak shape
   d. Missing transitions
      i. In the case of missing peptides or missing transitions, first, make a note in Skyline at the peptide level (right click peptide sequence, then "Edit Note" and supply description such as "no signal", "only 2 transitions detected", "not sure which peak is peptide", etc. The Skyline files on the NIST server may already contain "notes" indicating which peptides are weak or poorly detected. If you encounter additional peptides, consider creating an unscheduled method to target the few peptides that were undetectable. If they remain to be undetectable, please contact Birgit Schilling and Sue Abbatiello for further advice.

iii. Under Peptide Settings, set window to 4 minutes

1. Export the transition list in scheduled mode – heavy only, ~375 transitions with 4 min RT window. Double check that all 375 transitions are exported and added into the MS method appropriately.
2. Please set target cycle time to be approximately 1.5 seconds
   a. 4000 QTRAP users: please set to 2.0 sec
   b. 5500 QTRAP users: please set to 1.2 sec (1.5 if using Nanoflex)

c. Agilent ChipCube users: please adjust cycle time so that the minimum dwell time calculated in MassHunter is >10 msec.
d. Use the Autodwell feature of Waters software to determine dwell times based on two inputs: point-per-peak and average peak width.
e. Other platforms: please adjust cycle time such that you will get ~15 points across your chromatographic peak (baseline to baseline) and >10 msec dwell time minimum for all transitions.

3. Utilize the "scheduling" feature in Skyline which will show how many concurrent transitions there are in the most complex part of the gradient (highest number of concurrent transitions). Assess the transition distribution spanning the gradient evenly, aiming for a Gaussian-like distribution of the concurrent transitions in the Skyline scheduling view. This will help to see problems of early eluting hydrophilic peptides that might "bleed through", gradient problems might visually become evident, i.e., if everything elutes very early or very late or within a very small RT range.
4. Run two replicates of on-site digested/spiked sample Study9-2-A (which contains on site digested U-^{15}N-labeled protein plus operator-spiked heavy ^{13}C/^{15}N synthetic IS peptides in depleted plasma), in which the method monitors for only the 13C/15N heavy peptide forms with a 4 min RT window, and import into Skyline.
5. Ensure all 13C/15N peptide peaks are detected.

iv. Download a second, different Skyline template from the NIST server containing heavy (^{13}C/^{15}N), light (unlabeled) and U-^{15}N-labeled peptides (download the new template rather than adding in new label types into the existing heavy only template! Don't use Edit->Refine to add new label types) Note: appropriate corresponding Skyline templates for all three label types can be found on the FTP server in the Study 9.2 folder. Recommendation: Use one dedicated Skyline file with all three label types always for scheduling.
ftp://chemdata.nist.gov/verification_main_study9.2/Skyline_templates/

1. Import your last two acquired scheduled Study9-2-A samples into this newly downloaded Skyline template that includes all three label types
2. There should now be 1095 transitions, 125 peptides, 365 precursors
3. View the RT plot for Scheduling
4. Ensure the maximum number of concurrent transitions throughout the gradient is < 100
5. If there are incidents of concurrent transitions >100 anywhere in the gradient, contact CPTAC VWG members for assistance
6. Set the RT window for 2 minutes (Settings, Peptide Settings, Prediction Tab).

5. Export all transitions (~1095) in Scheduled mode, with 2 min RT window. Check that your two (4 min)-scheduled Study9-2-A samples that you have imported above into your all inclusive Skyline template (3 label forms) do not show much RT drift, and use the second, most recent Study9-2-A run for scheduling (with a 2 min scheduling window). Double check that all 1095 transitions are exported and added into the MS method

appropriately (i.e., the total number of transitions is correct and that each light, $^{13}C/^{15}N$, U-^{15}N set have the same retention times for all 9 transitions). Acquire 1- 4 replicates of sample Study9-2 A with the scheduled LC-MRM-MS method (now using the 2 min scheduling window)

i. Import data back into Skyline and observe any shift in retention times
ii. Use RT graph, peptide replicate view
iii. If peaks shift by > 30 seconds (0.5 minutes), do not proceed and contact CPTAC VWG members for assistance
iv. Look at data to make sure peak apexes are in or near the center of the RT window and not getting cut off during detection.
v. The Study 9-2-A sample contains heavy peptide signal ($^{13}C^{15}N$ peptides), but also U-^{15}N peptides that were generated from your digestion on-site. Look for the U-^{15}N peptide signal and if you don't see the U-^{15}N peptide signal possibly something was unusual with the performed digestion. In such case, please pause and assess and troubleshoot. The last 7 proteins in the Skyline file are for the digestion controls. You should see consistent peak area ratios (light : $^{13}C/^{15}N$) for the peptides across your standards. If these vary greatly, also pause and troubleshoot.

6. Continue acquiring Study 9-2 samples according to the sample chart shown below.

3. Important additional Notes for Sample Preparation and Acquisitions:

a) The four replicate concentration points in Study 9.1 and here in Study 9.2 will be acquired in **4x singlicate curves** (with blanks and SSS in between to avoid carryover). Also note each non-digested Study 9.2 singlicate concentration point was prepared individually at the central preparation site (Vanderbilt), so for each concentration point there will be 3 tubes supplied (the 3rd replicate supplied will be split into to technical replicates after reconstitution, and will be acquired twice on the LC-MRM-MS platform as curve 3 and curve 4, also see Scheme 1 above).

b) **After digestion, desalting, and reconstitution (including adding in the heavy synthetic peptides, as described below)**, take a 25 µl aliquot of each reconstituted sample for immediate LC-MRM-MS acquisition (the total volume per sample after digestion, desalting, lyophilizing and reconstitution is **225 µL**). There will be a remainder of each sample of 200 µl, aliquot this remainder into 50 µl aliquots and freeze those at -80 ºC (the latter is very important, please freeze the non-used digested reconstituted sample in aliquots so one can retrieve them later for possible reruns).

c) **Adding Calibration Curve Samples and Blinded Samples into Autosampler Vials:** Add one Calibration Curve and corresponding Blinded Samples at a time into the autosampler. Transfer a 25 µL aliquot from each Calibration Curve concentration point tube per singlicate curve (as described above) into an autosampler vial, and store the remaining 50 µL aliquots generated in the -80 ºC freezer (there should be 4x 50 µL aliquots leftover per concentration point and singlicate curve / and half of that for the split 3rd set). Make sure to finish each singlicate curve (including initial data analysis and review of data points for quality, or for retention time drift of peaks outside the RT scheduling window) before starting the next singlicate curve. If data points need to be rerun from the current singlicate curve use remaining sample that is still in autosampler

vials or if multiple reruns are needed use one of the aliquots that was transferred into the freezer as was described above. At the beginning of a singlicate curve, and as blank in between blinded samples transfer 25 µL of the 9.2 A aliquot into an autosampler vial.

d) **In Case of Acquisition Problems and Re-runs of Samples:** In case there are problems with the data acquisition or RT drift, and possible reruns that may become necessary, please maintain the run number at the end of the filename to keep track of the run order. The run number should agree with the chronological order in which the samples were analyzed on the LC-MRM-MS. In addition, please re-run the blank 9.2-A sample prior to any re-run, and please re-run from lowest to highest concentration for the calibration curve samples.

e) **Binded Samples**

i. There are three sets of three Blinded samples (each provided in 35 µl aliquot, of which 25 µl are digested), with Set 3 being split into two technical replicates T1 and T2 after digestion and desalting:

1. First singlicate: 9-2-01Blinded to 9-2-03Blinded
2. Second singlicate: 9-2-04Blinded to 9-2-06Blinded
3. Third singlicate: 9-2-07-T1Blinded to 9-2-09-T1Blinded
4. Fourth singlicate: 9-2-07-T2Blinded to 9-2-09-T2Blinded

ii. For each singlicate: the three blinded samples will be run **twice**, i.e., as runs 9-2-01aBlinded and 9-2-01bBlinded, see runorder.

iii. Blinded samples will be run with 1 wash run (10 fmol 6-protein mix) and 1 blank run in between. The blank run consists of Sample 9-2-A, that was digested on-site and which contains no unlabeled peptides, only the $^{13}C/^{15}N$ IS peptides and U-^{15}N-labeled peptide equivalents in depleted plasma.

iv. For Blinded Samples use Study 9-2 gradient (Table 2a) and "Study 9 scheduled MRM-MS, light and heavy" MS-method. For Wash Samples use Wash gradient (Table 2c) and "Study 9S, SSS"-adjusted MS-method. For Blank Samples use Study 9-2 gradient (Table 2a) and "Study 9 scheduled MRM-MS, light and heavy" MS-method. Blanks (in between blinded samples) can be injected from the same autosampler vial

7. **Fresh Preparation of System Suitability Samples (SSS) for Each Singlicate Curve:** For each Singlicate Calibration Curve a fresh autosampler vial of the SSS will should be prepared to prevent degradation of the sample (note in a notebook when fresh SSS are added).
8. **Please adhere closely to sample naming as defined in the run order** (with chronological runorder number at the end).
9. **Note that System Suitability samples (SSS) indicate which singlicate curve they belong to:** S1 for Singlicate Curve 1, S2 for Singlicate Curve 2, S3 for Singlicate Curve 3, and S4 for Singlicate Curve 4.

Please remember: there are 3 different gradients to be used in the run orders below:

- **Study 9S** gradient for System Suitability Samples
- **Study 9-2** gradient for the main study (all concentration curve points as well as blinded samples)
- **Study 9 wash** gradient (in between blinded samples)

Gradients are described in detail in Tables 2a-c, and 3a-c for Waters UPLC (see below)

First singlicate curve plus blinded samples (01-03blinded)

Run Number	Number of injections	Sample Description	Filename	Method	Notes
1	1	SSS, Michrom Mix	9-2_SiteX_SSS_run_001	Study 9S (SSS)	Column Conditioning
2	1	SSS, Michrom Mix	9-2_SiteX_SSS_run_002	Study 9S (SSS)	
3	1	SSS, Michrom Mix	9-2_SiteX_SSS_run_003	Study 9S (SSS)	
4	1	SSS, Michrom Mix	9-2_SiteX_SSS_run_004	Study 9S (SSS)	
5	1	SSS, Michrom Mix	9-2_SiteX_SSS_run_005	Study 9S (SSS)	
6	1	Study9-2 Heavy Only	9-2_SiteX_heavy_method01_run_006	Study 9 method 01	For scheduling
7	1	Study9-2 Heavy Only	9-2_SiteX_heavy_method02_run_007	Study 9 method 02	
8	1	Study9-2 Heavy Only	9-2_SiteX_heavy_method03_run_008	Study 9 method 03	
9	1	Study9-2 Heavy Only	9-2_SiteX_heavy_method04_run_009	Study 9 method 04	
10	1	Study9-2 Heavy Only	9-2_SiteX_heavy_method05_run 010	Study 9 method 05	
11	1	Study9-2 Heavy Only	9-2_SiteX_heavy_method06_run_011	Study 9 method 06	
12-16	5	SSS, Michrom Mix	9-2_SiteX_SSS_S1_run_012 9-2_SiteX_SSS_S1_run_013 9-2_SiteX_SSS_S1_run_014 9-2_SiteX_SSS_S1_run_015 9-2_SiteX_SSS_S1_run_016	Study 9S (SSS)	At least 3 SSS samples and if necessary place holder
17	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_sMRM_run_017	Study 9 scheduled MRM-MS, heavy only	Scheduled run, 4 min RT window
18	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_sMRM_run_018	Study 9 scheduled MRM-MS, heavy only	
19	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_sMRM_run_019	Study 9 scheduled MRM-MS, light heavy, and U-^{15}N	Scheduled run, 2 min RT window
20	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_sMRM_run_020	Study 9 scheduled MRM-MS, light heavy, and U-^{15}N	
21	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_sMRM_run_021	Study 9 scheduled MRM-MS, light heavy, and U-^{15}N	
22	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_sMRM_run_022	Study 9 scheduled MRM-MS, light heavy, and U-^{15}N	

23	1	Study9-2 A1, curve proteins in plasma	9-2_SiteX_A1_CalCurve_run_023	Study 9 scheduled MRM-MS, light heavy, and U-^{15}N	Calibration Curve
24	1	Study9-2 B1, curve proteins in plasma	9-2_SiteX_B1_CalCurve_run_024	Study 9 scheduled MRM-MS, light heavy, and U-^{15}N	
25	1	Study9-2 C1	9-2_SiteX_C1_CalCurve_run_025	Study 9 scheduled MRM-MS, light, heavy, U-^{15}N "Study9 sMRM"	CalCurve
26	1	Study9-2 D1	9-2_SiteX_D1_CalCurve_run_026	Study9 sMRM	CalCurve
27	1	SSS	9-2_SiteX_SSS_S1_run_027	Study 9S (SSS)	System Suitability
28	1	Study9-2 E1	9-2_SiteX_E1_CalCurve_run_028	Study9 sMRM	CalCurve
29	1	Study9-2 F1	9-2_SiteX_F1_CalCurve_run_029	Study9 sMRM	CalCurve
30	1	Study9-2 G1	9-2_SiteX_G1_CalCurve_run_030	Study9 sMRM	CalCurve
31	1	Study9-2 H1	9-2_SiteX_H1_CalCurve_run_031	Study9 sMRM	CalCurve
32	1	Study9-2 QC1	9-2_SiteX_QC1_CalCurve_run_032	Study9 sMRM	QC
33	1	Study9-2 I1	9-2_SiteX_I1_CalCurve_run_033	Study9 sMRM	CalCurve
34	1	Study9-2 J1	9-2_SiteX_J1_CalCurve_run_034	Study9 sMRM	CalCurve
35	1	Study9-2 wash	9-2_SiteX_wash_run_35	Study 9 wash	
36	1	SSS	9-2_SiteX_SSS_S1_run_036	Study 9S (SSS)	System suitability
37	1	SSS	9-2_SiteX_SSS_S1_run_037	Study 9S (SSS)	System suitability
38	1	SSS	9-2_SiteX_SSS_S1_run_038	Study 9S (SSS)	System suitability
39	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank_run_039	Study 9 scheduled MRM-MS, light and heavy	
40	1	Study9-2 01blinded-Sample	9-2_SiteX_01a_blinded_run_040	Study 9 scheduled MRM-MS, light and heavy	Blinded
41	1	Study9-2 wash	9-2_SiteX_wash_run_041	Study 9 wash	
42	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_042	Study 9 scheduled MRM-MS, light and heavy	
43	1	Study9-2 02blinded	9-2_SiteX_02a_blinded_run_043	Study 9 scheduled	Blinded

				MRM-MS, light and heavy	
44	1	Study9-2 wash	9-2_SiteX_wash_run_044	Study 9 wash	
45	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_045	Study 9 scheduled MRM-MS, light and heavy	
46	1	SSS	9-2_SiteX_SSS_S1_run_046	Study 9S (SSS)	System suitability
47	1	Study9-2 03blinded	9-2_SiteX_03a_blinded_run_047	Study 9 scheduled MRM-MS, light and heavy	Blinded
48	1	Study9-2 wash	9-2_SiteX_wash_run_048	Study 9 wash	
49	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_049	Study 9 scheduled MRM-MS, light and heavy	
50	1	Study9-2 01blinded-Sample	9-2_SiteX_01b_blinded_run_050	Study 9 scheduled MRM-MS, light and heavy	Blinded
51	1	Study9-2 wash	9-2_SiteX_wash_run_051	Study 9 wash	
52	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_052	Study 9 scheduled MRM-MS, light and heavy	
53	1	Study9-2 02blinded	9-2_SiteX_02b_blinded_run_053	Study 9 scheduled MRM-MS, light and heavy	Blinded
54	1	Study9-2 wash	9-2_SiteX_wash_run_054	Study 9 wash	
55	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_055	Study 9 scheduled MRM-MS, light and heavy	
56	1	SSS	9-2_SiteX_SSS_S1_run_056	Study 9S (SSS)	System suitability
57	1	Study9-2 03blinded	9-2_SiteX_03b_blinded_run_057	Study 9 scheduled MRM-MS, light and heavy	Blinded
58	1	Study9-2 wash	9-2_SiteX_wash_run_058	Study 9 wash	

59	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_059	Study 9 scheduled MRM-MS, light and heavy	

Second singlicate curve plus blinded samples (04-06blinded)

60-62	3	SSS, Michrom Mix	9-2_SiteX_SSS_S2_run_060 9-2_SiteX_SSS_S2_run_061 9-2_SiteX_SSS_S2_run_062	Study 9S (SSS)	3 SSS samples
63	1	Study9-2 A2, proteins in plasma	9-2_SiteX_A2_CalCurve_run_063	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window, Calibration Curve
64	1	Study9-2 B2	9-2_SiteX_B2_CalCurve_run_064	Study 9 scheduled MRM-MS, light and heavy	
65	1	Study9-2 C2	9-2_SiteX_C2_CalCurve_run_065	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
66	1	Study9-2 D2	9-2_SiteX_D2_CalCurve_run_066	Study9 sMRM	CalCurve
67	1	SSS	9-2_SiteX_SSS_S2_run_067	Study 9S (SSS)	System Suitability
68	1	Study9-2 E2	9-2_SiteX_E2_CalCurve_run_068	Study9 sMRM	CalCurve
69	1	Study9-2 F2	9-2_SiteX_F2_CalCurve_run_069	Study9 sMRM	CalCurve
70	1	Study9-2 G2	9-2_SiteX_G2_CalCurve_run_070	Study9 sMRM	CalCurve
71	1	Study9-2 H2	9-2_SiteX_H2_CalCurve_run_071	Study9 sMRM	CalCurve
72	1	Study9-2 QC2	9-2_SiteX_QC2_CalCurve_run_072	Study9 sMRM	QC
73	1	Study9-2 I2	9-2_SiteX_I2_CalCurve_run_073	Study9 sMRM	CalCurve
74	1	Study9-2 J2	9-2_SiteX_J2_CalCurve_run_074	Study9 sMRM	CalCurve
75	1	Study9-2 wash	9-2_SiteX_wash_run_075	Study 9 wash	
76	1	SSS	9-2_SiteX_SSS_S2_run_076	Study 9S (SSS)	System suitability
77	1	SSS	9-2_SiteX_SSS_S2_run_077	Study 9S (SSS)	System suitability
78	1	SSS	9-2_SiteX_SSS_S2_run_078	Study 9S (SSS)	System suitability
79	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank_run_079	Study 9 scheduled MRM-MS, light	

				and heavy	
80	1	Study9-2 04blinded-Sample	9-2_SiteX_04a_blinded_run_080	Study 9 scheduled MRM-MS, light and heavy	Blinded
81	1	Study9-2 wash	9-2_SiteX_wash_run_081	Study 9 wash	
82	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_082	Study 9 scheduled MRM-MS, light and heavy	
83	1	Study9-2 05blinded	9-2_SiteX_05a_blinded_run_083	Study 9 scheduled MRM-MS, light and heavy	Blinded
84	1	Study9-2 wash	9-2_SiteX_wash_run_084	Study 9 wash	
85	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_085	Study 9 scheduled MRM-MS, light and heavy	
86	1	SSS	9-2_SiteX_SSS_S2_run_086	Study 9S (SSS)	System suitability
87	1	Study9-2 06blinded	9-2_SiteX_06a_blinded_run_087	Study 9 scheduled MRM-MS, light and heavy	Blinded
88	1	Study9-2 wash	9-2_SiteX_wash_run_088	Study 9 wash	
89	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_089	Study 9 scheduled MRM-MS, light and heavy	
90	1	Study9-2 04blinded-Sample	9-2_SiteX_04b_blinded_run_090	Study 9 scheduled MRM-MS, light and heavy	Blinded
91	1	Study9-2 wash	9-2_SiteX_wash_run_091	Study 9 wash	
92	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_092	Study 9 scheduled MRM-MS, light and heavy	
93	1	Study9-2 05blinded	9-2_SiteX_05b_blinded_run_093	Study 9 scheduled MRM-MS, light and heavy	Blinded
94	1	Study9-2 wash	9-2_SiteX_wash_run_094	Study 9 wash	

95	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_095	Study 9 scheduled MRM-MS, light and heavy	
96	1	SSS	9-2_SiteX_SSS_S2_run_096	Study 9S (SSS)	System suitability
97	1	Study9-2 06blinded	9-2_SiteX_06b_blinded_run_097	Study 9 scheduled MRM-MS, light and heavy	Blinded
98	1	Study9-2 wash	9-2_SiteX_wash_run_098	Study 9 wash	
99	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_099	Study 9 scheduled MRM-MS, light and heavy	

Third singlicate curve plus blinded samples (07-09blinded), first technical replicate 3-T1

100-102	3	SSS, Michrom Mix	9-2_SiteX_SSS_S3_run_100 9-2_SiteX_SSS_S3_run_101 9-2_SiteX_SSS_S3_run_102	Study 9S (SSS)	3 SSS samples
103	1	Study9-2 A3-T1, proteins in plasma	9-2_SiteX_A3-T1_CalCurve_run_103	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window, Calibration Curve
104	1	Study9-2 B3-T1	9-2_SiteX_B3-T1_CalCurve_run_104	Study 9 scheduled MRM-MS, light and heavy	
105	1	Study9-2 C3-T1	9-2_SiteX_C3-T1_CalCurve_run_105	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
106	1	Study9-2 D3-T1	9-2_SiteX_D3-T1_CalCurve_run_106	Study9 sMRM	CalCurve
107	1	SSS	9-2_SiteX_SSS_S3_run_107	Study 9S (SSS)	System Suitability
108	1	Study9-2 E3-T1	9-2_SiteX_E3-T1_CalCurve_run_108	Study9 sMRM	CalCurve
109	1	Study9-2 F3-T1	9-2_SiteX_F3-T1_CalCurve_run_109	Study9 sMRM	CalCurve
110	1	Study9-2 G3-T1	9-2_SiteX_G3-T1_CalCurve_run_110	Study9 sMRM	CalCurve

111	1	Study9-2 H3-T1	9-2_SiteX_H3-T1_CalCurve_run_111	Study9 sMRM	CalCurve
112	1	Study9-2 QC3	9-2_SiteX_QC3_CalCurve_run_112	Study9 sMRM	QC
113	1	Study9-2 I3-T1	9-2_SiteX_I3-T1_CalCurve_run_113	Study9 sMRM	CalCurve
114	1	Study9-2 J3-T1	9-2_SiteX_J3-T1_CalCurve_run_114	Study9 sMRM	CalCurve
115	1	Study9-2 wash	9-2_SiteX_wash_run_115	Study 9 wash	
116	1	SSS	9-2_SiteX_SSS_S3_run_116	Study 9S (SSS)	System suitability
117	1	SSS	9-2_SiteX_SSS_S3_run_117	Study 9S (SSS)	System suitability
118	1	SSS	9-2_SiteX_SSS_S3_run_118	Study 9S (SSS)	System suitability
119	1	Study9-2 A-T1, IS peptides in plasma	9-2_SiteX_A-T1_blank_run_119	Study 9 scheduled MRM-MS, light and heavy	
120	1	Study9-2 07-T1blinded-Sample	9-2_SiteX_07a-T1blinded_run_120	Study 9 scheduled MRM-MS, light and heavy	Blinded
121	1	Study9-2 wash	9-2_SiteX_wash_run_121	Study 9 wash	
122	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A _blank _run_122	Study 9 scheduled MRM-MS, light and heavy	
123	1	Study9-2 08-T1blinded	9-2_SiteX_08a-T1blinded_run_123	Study 9 scheduled MRM-MS, light and heavy	Blinded
124	1	Study9-2 wash	9-2_SiteX_wash_run_124	Study 9 wash	
125	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_125	Study 9 scheduled MRM-MS, light and heavy	
126	1	SSS	9-2_SiteX_SSS_S3_run_126	Study 9S (SSS)	System suitability
127	1	Study9-2 09-T1-blinded	9-2_SiteX_09a-T1blinded_run_127	Study 9 scheduled MRM-MS, light and heavy	Blinded
128	1	Study9-2 wash	9-2_SiteX_wash_run_128	Study 9 wash	
129	1	Study9-2 A, IS peptides in	9-2_SiteX_A_blank _run_129	Study 9 scheduled	

		plasma		MRM-MS, light and heavy	
130	1	Study9-2 07-T1blinded-Sample	9-2_SiteX_07b-T1blinded_run_130	Study 9 scheduled MRM-MS, light and heavy	Blinded
131	1	Study9-2 wash	9-2_SiteX_wash_run_131	Study 9 wash	
132	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A _blank _run_132	Study 9 scheduled MRM-MS, light and heavy	
133	1	Study9-2 08-T1blinded	9-2_SiteX_08b-T1blinded_run_133	Study 9 scheduled MRM-MS, light and heavy	Blinded
134	1	Study9-2 wash	9-2_SiteX_wash_run_134	Study 9 wash	
135	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_135	Study 9 scheduled MRM-MS, light and heavy	
136	1	SSS	9-2_SiteX_SSS_S3_run_136	Study 9S (SSS)	System suitability
137	1	Study9-2 09-T1-blinded	9-2_SiteX_09b-T1blinded_run_137	Study 9 scheduled MRM-MS, light and heavy	Blinded
138	1	Study9-2 wash	9-2_SiteX_wash_run_138	Study 9 wash	
139	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_139	Study 9 scheduled MRM-MS, light and heavy	

Technical replicate T2 of third singlicate curve plus blinded samples (07-09blinded, technical replicate2, T2)

140-142	3	SSS, Michrom Mix	9-2_SiteX_SSS_S4_run_140 9-2_SiteX_SSS_S4_run_141 9-2_SiteX_SSS_S4_run_142	Study 9S (SSS)	3 SSS samples
143	1	Study9-2 A3_T2, proteins in plasma	9-2_SiteX_A3_T2_CalCurve_run_143	Study 9 scheduled MRM-MS, light and heavy	Scheduled run, 2 min RT window,

144	1	Study9-2 B3_T2	9-2_SiteX_B3_T2_CalCurve_run_144	Study 9 scheduled MRM-MS, light and heavy	Calibration Curve
145	1	Study9-2 C3_T2	9-2_SiteX_C3_T2_CalCurve_run_145	Study 9 scheduled MRM-MS, light and heavy, "Study9 sMRM"	CalCurve
146	1	Study9-2 D3_T2	9-2_SiteX_D3_T2_CalCurve_run_146	Study9 sMRM	CalCurve
147	1	SSS	9-2_SiteX_SSS_S4_run_147	Study 9S (SSS)	System Suitability
148	1	Study9-2 E3_T2	9-2_SiteX_E3_T2_CalCurve_run_148	Study9 sMRM	CalCurve
149	1	Study9-2 F3_T2	9-2_SiteX_F3_T2_CalCurve_run_149	Study9 sMRM	CalCurve
150	1	Study9-2 G3_T2	9-2_SiteX_G3_T2_CalCurve_run_150	Study9 sMRM	CalCurve
151	1	Study9-2 H3_T2	9-2_SiteX_H3_T2_CalCurve_run_151	Study9 sMRM	CalCurve
152	1	Study9-2 QC4	9-2_SiteX_QC4_CalCurve_run_152	Study9 sMRM	QC
153	1	Study9-2 I3_T2	9-2_SiteX_I3_T2_CalCurve_run_153	Study9 sMRM	CalCurve
154	1	Study9-2 J3_T2	9-2_SiteX_J3_T2_CalCurve_run_154	Study9 sMRM	CalCurve
155	1	Study9-2 wash	9-2_SiteX_wash_run_155	Study 9 wash	
156	1	SSS	9-2_SiteX_SSS_S4_run_156	Study 9S (SSS)	System suitability
157	1	SSS	9-2_SiteX_SSS_S4_run_157	Study 9S (SSS)	System suitability
158	1	SSS	9-2_SiteX_SSS_S4_run_158	Study 9S (SSS)	System suitability
159	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank_run_159	Study 9 scheduled MRM-MS, light and heavy	
160	1	Study9-2 07_T2blinded-Sample	9-2_SiteX_07a_T2blinded_run_160	Study 9 scheduled MRM-MS, light and heavy	Blinded
161	1	Study9-2 wash	9-2_SiteX_wash_run_161	Study 9 wash	
162	1	Study9-2 A, IS peptides in plasma	9-2_SiteX_A_blank _run_162	Study 9 scheduled MRM-MS, light and heavy	
163	1	Study9-2 08_T2blinded	9-2_SiteX_08a_T2blinded_run_163	Study 9 scheduled MRM-MS, light and heavy	Blinded

164	**1**	**Study9-2 wash**	**9-2_SiteX_wash_run_164**	**Study 9 wash**	
165	**1**	**Study9-2 A, IS peptides in plasma**	**9-2_SiteX_A_blank _run_165**	**Study 9 scheduled MRM-MS, light and heavy**	
166	**1**	**SSS**	**9-2_SiteX_SSS_S4_run_166**	**Study 9S (SSS)**	**System suitability**
167	**1**	**Study9-2 09_T2blinded**	**9-2_SiteX_09a_T2blinded_run_167**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
168	**1**	**Study9-2 wash**	**9-2_SiteX_wash_run_168**	**Study 9 wash**	
169	**1**	**Study9-2 A, IS peptides in plasma**	**9-2_SiteX_A_blank _run_169**	**Study 9 scheduled MRM-MS, light and heavy**	
170	**1**	**Study9-2 07_T2blinded-Sample**	**9-2_SiteX_07b_T2blinded_run_170**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
171	**1**	**Study9-2 wash**	**9-2_SiteX_wash_run_171**	**Study 9 wash**	
172	**1**	**Study9-2 A, IS peptides in plasma**	**9-2_SiteX_A_blank _run_172**	**Study 9 scheduled MRM-MS, light and heavy**	
173	**1**	**Study9-2 08_T2blinded**	**9-2_SiteX_08b_T2blinded_run_173**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
174	**1**	**Study9-2 wash**	**9-2_SiteX_wash_run_174**	**Study 9 wash**	
175	**1**	**Study9-2 A, IS peptides in plasma**	**9-2_SiteX_A_blank _run_175**	**Study 9 scheduled MRM-MS, light and heavy**	
176	**1**	**SSS**	**9-2_SiteX_SSS_S4_run_176**	**Study 9S (SSS)**	**System suitability**
177	**1**	**Study9-2 09_T2blinded**	**9-2_SiteX_09b_T2blinded_run_177**	**Study 9 scheduled MRM-MS, light and heavy**	**Blinded**
178	**1**	**Study9-2 wash**	**9-2_SiteX_wash_run_178**	**Study 9 wash**	
179	**1**	**Study9-2 A, IS peptides in**	**9-2_SiteX_A_blank _run_179**	**Study 9 scheduled**	

		plasma		**MRM-MS, light and heavy**	
180	**1**	**SSS**	**9-2_SiteX_SSS_S4_run_180**	**Study 9S (SSS)**	**System suitability**
181	**1**	**SSS**	**9-2_SiteX_SSS_S4_run_181**	**Study 9S (SSS)**	**System suitability**
182	**1**	**SSS**	**9-2_SiteX_SSS_S4_run_182**	**Study 9S (SSS)**	**System suitability**

1. **Data Analysis**
    a. All data should be analyzed in "real time," or as close to its acquisition time as possible to catch the chance of peaks drifting outside the 2 min RT windows.
    b. Data analysis must be conducted through Skyline using templates provided for each instrument platform
    c. Once data are imported, check integration of all peaks
        i. Integrate transitions with the same start and stop time: Under "Settings" in Skyline, enable "Integrate All"
        ii. Integrate light, heavy, and U-^{15}N peptides with the same start and stop time
        iii. If an interference is present, document the transition-level note for that transition
    d. Export data for submission to VWG statisticians using the report template provided (note the new Study 9.2 Skyline report template is slightly different from the 9.1 template, and can be found on the NIST server, ftp://chemdata.nist.gov/verification_main_study9.2/Skyline_templates/)
    e. Under Settings, click on "custom annotation" and make sure that all settings are selected.
        i. If a transition, precursor, or peptide is deemed unusable because of a bad injection, loss of electrospray or another explainable reason and the sample is re-run, please click the "do not use" box for the sample that is to be excluded from subsequent analysis for LOD/LOQ.

Even if a specific replicate needs to be rerun, the original and new (rerun) replicate are to both be imported into Skyline, and "custom annotation" capabilities of Skyline should be used to annotate what data points to use for statistical analysis.

4. Trouble Shooting Section

b. Symptom: No data (missing peaks) or poor quality peaks for Study 9 peptides

i. Look at pressure trace to make sure sample (not air) was injected onto column.

ii. Open up the last SSS acquired before file with missing peaks

1. In Skyline or in vendor specific software

iii. Are 22 peaks present?

1. Yes: go on to point iv.
2. No: identify which peaks are missing
    a. Check pressure trace to make sure air was not injected onto column and that column pressure isn't too high
    b. Is there enough sample in all vials (sample vials and reagent vials)?

iv. Are peak shapes symmetric and well defined as opposed to mis-shapen and jagged?

1. Yes: go on to point v.
2. No: check ESI tip of column, clean if necessary.
    a. Also check pressure trace to make sure pressure is not too high.

v. Is column pressure too high?

1. Compare column pressure of bad runs to previous runs in the sample list
    a. If pressure is too high, remove column and clean tip
        i. Wipe tip with gloved hand, wet with water and wipe again. Check tip under microscope if possible.

ii. If this does not work, check for blockages in transfer lines closest to column and work backwards to LC.
iii. If this does not work, consider replacing column.

vi. Is column pressure too low?

1. If pressure is too low, check all connections between column and LC.

vii. Are flow rates calibrated correctly? (potentially check or recalibrate flow rates)
viii. Are samples being picked up properly from the autosampler vials?

c. Symptom: Retention Time Drift

i. Observe RT shift of the SSS samples in a single Skyline document

1. Does RT shift in one direction or randomly?

ii. If in one direction, are the peaks migrating out of the 2 min RT window for the Study 9 method? Solution: Re-scheduling because of shifting retention times

1. Re-run Study 9-2_A with the "heavy only" scheduled method that has a 4 minute RT window.
2. Import data into an empty Skyline template for Study 9 peptides and export a new scheduled method based on the RT data for light and heavy peptides (750 transitions).
3. Keep close eye on RT shifts.
4. Consider running SSS runs at points when you would not be able to check the RT shift and if peaks are in danger of shifting out of RT window (like overnight).

d. **If any concentration points must be re-run, it is important to follow this procedure**:

i. Run SSS sample to ensure system is working properly. Look for smooth, symmetrical peaks with stable retention times.
ii. Inject Sample Study9-2_A before re-running a calibration point to make sure there is little carryover.
iii. Follow with another SSS run so that you have time to analyze the data before running the next concentration point.

HPLC Chromatography Conditions for Studies 9-2:

Individual CPTAC sites are expected to implement these HPLC conditions for the duration of the study. Prepacked New Objective HPLC Columns are provided.

- HPLC-plumbing: DIRECT INJECTION
- Sample Loop: 1 µL sample loop
- Columns for AB SCIEX and Thermo HPLC systems: (New Objective custom-packed as previously shipped to all sites): PicoFrit 75 µm ID / 10 µm tip (ReproSil-Pur C18-AQ, 3µm, 120Å, length of 12 cm)
- Columns for Waters UPLC systems: a Waters specific column will be used (1.7µm BEH130 C18, 75 µm X 150 mm column)
- Columns for Agilent ChipCube systems: NanoChip
- Mobile phases: (A) 0.1% Formic acid (v/v); (B) 90% Acetonitrile / 0.1% Formic acid (v/v)

- Flow rate: 300 nL/min
- Injection volume: 1 µL on column (full loop injection with overloading the loop with 2 µL)
- Loop for Eksigent LC: 1 µL PEEKsil loop provided in sample kit, 100 µm ID PEEKsil.
- Injection Amount: ~0.5 µg total protein on-column
- Gradient: for details see below, **NOTE: Study 9S system suitability has a slightly different gradient than the new Study 9-2 gradient. Please use the appropriate gradient for these different applications.**

Table 1: Eksigent/Tempo Notes: Autosampler set-up, direct injection mode (representative example)

Autosampler Program with Standard Injection*

#	Function	Command		
1	Output	1-Off		
2	Output	2-Off		
3	Valve	Injector Load		
4**	Aspirate	10 µL Reagent-1	Speed:1	Height:5
5	Aspirate	2 µL Sample	Speed:1	Height:2
6**	Aspirate	2.3 µL Reagent-1	Speed:1	Height:5
7	Output	2-On		
8	Valve	Injector Inject		
9	Dispense	14.3 µL Waste	Speed:5	Height:0
10 **	Needle Wash	200 µL		
11	End			

NOTE: Different gradients are used for Study 9-2 calibration curve (Study 9-2 gradient) vs. system suitability samples (Study 9S gradient) vs. Wash gradient used in between blinded samples (Study 9 Wash gradient).

Table 2a. 9-2 HPLC gradient for all analyses of 125 target synthetic peptides.

Time (min)	%A	%B	Flow Rate (nL/min)
0	97	3	300
5	97	3	300
8	93	7	300
35	75	25	300
42	60	40	300
45	10	90	300
49	10	90	300
50	97	3	300
80	97	3	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 90% acetonitrile/0.1% formic acid (v/v) in water

Table 2b. 9S HPLC gradient to be used for System Suitability Sample (Michrom 6 protein mix).

Time (min)	%A	%B	Flow Rate (nL/min)
0	97	3	300
5	97	3	300
8	85	15	300
42	65	35	300
45	10	90	300
49	10	90	300
50	97	3	300
80	97	3	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 90% acetonitrile/0.1% formic acid (v/v) in water

Table 2c. 9-Wash HPLC gradient to be used for washing the column in between Blinded Samples.

Time (min)	%A	%B	Flow Rate (nL/min)
0	97	3	300
15	10	90	300
16	97	3	300
31	10	90	300
32	97	3	300
62	97	3	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 90% acetonitrile/0.1% formic acid (v/v) in water

NOTE: Total peptide amount greater than 1 µg injected onto nanoLC columns can result in poor chromatographic peak shape and poor reproducibility from run to run. The MARS-14 depleted plasma samples (Samples 9-2-A through 9-2-J, and 9-2-Blank) have been diluted such that a 1 µL injection results in approximately 0.5 µg of total protein on-column. Therefore, they should be analyzed without any additional dilution.

For Waters operators only:

Operators with Waters Aquity UPLC will use a slighly adjusted gradient, due to the use of Mobile phase B: 100% acetonitrile (v/v)/0.1% formic acid in water (v/v) only for Waters UPLC.

Adjusted Table 3a. for Waters UPLC only. Waters 9-2 HPLC gradient for all analyses of 125 target synthetic peptides.

Time (min)	%A	%B	Flow Rate (nL/min)
0	97.3	2.7	300
5	97.3	2.7	300
8	93.7	6.3	300
35	77.5	22.5	300
42	64	36	300
45	19	81	300
49	19	81	300
50	97.3	2.7	300
80	97.3	2.7	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 100% acetonitrile/0.1% formic acid (v/v) in water

Adjusted Table 3b. for Waters UPLC only. Waters 9S HPLC gradient to be used for System Suitability Sample (Michrom 6-protein mix).

Time (min)	%A	%B	Flow Rate (nL/min)
0	97.3	2.7	300
5	97.3	2.7	300
8	86.5	13.5	300
42	68.5	31.5	300
45	19	81	300
49	19	81	300
50	97.3	2.7	300
80	97.3	2.7	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 100% acetonitrile/0.1% formic acid (v/v) in water

Adjusted Table 3c. for Waters UPLC only. Waters 9-Wash HPLC gradient to be used for washing the column in between Blinded Samples.

Time (min)	%A	%B	Flow Rate (nL/min)
0	97.3	2.7	300
15	19	81	300
16	97.3	2.7	300
31	19	81	300
32	97.3	2.7	300
62	97.3	2.7	300

Mobile phase A: 0.1% formic acid (v/v) in water
Mobile phase B: 100% acetonitrile/0.1% formic acid (v/v) in water

Appendix A

Preparation of buffer solutions for digestion:
I. Chemical Reagents for Sample Preparation and Recommended Source

The following chemicals are NOT provided in the sample kits:

1. Tris Base – Sigma
2. Water, HPLC grade
3. Acetonitrile, HPCL grade
4. Formic acid
5. Hydrochloric acid (12M, for adjusting pH of Tris solution)

The following chemicals are <u>included</u> in the sample kits:

1. Urea – SigmaUltra
2. 1,4-Dithiothreitol [DTT]- Pierce No-Weight Format, (pre-weighed in 7.7 mg aliquots to be used fresh for each experiment.
3. Iodoacetamide – Sigma
4. Promega Trypsin Gold (2 vials)
5. LysC
6. $^{13}C/^{15}N$ IS peptide mixture supplied at 500 fmol/uL in 30% acetonitrile/0.1% formic acid.

II. Digestion Reagent Preparation for Plasma/target Protein Digestions

A. 1 M Tris, pH 8.0 – 250 mL

1. To a 500 mL beaker, add 30.3 g solid Tris-base and add 150 mL deionized water and stir until dissolved.
2. Adjust pH of solution to 8.0 with concentrated HCl (12 M).
3. Transfer solution to 250 mL or 500 mL graduated cylinder and bring volume to 250mL with deionized water.

B. 9M urea, 100 mM Tris, pH 8.0 Stock - 50 mL
(This solution must be prepared fresh for each process replicate.)

1. To a 100 mL beaker, add 27 g solid urea
2. Add 15 mL of water and 5 mL of 1 M Tris, pH 8.0 (Reagent A).
3. Add stir bar and place beaker in larger beaker of warm water. Stir until dissolved. Keep temperature at or below 37 °C. Do not overheat!
4. Measure pH and adjust to 8.0 if necessary.
5. Transfer to 50 or 100 mL graduated cylinder and bring volume to 50 mL with deionized water.

C. 100 mM Tris, pH 8.0 – 100 mL

1. Add 10 mL of 1 M Tris pH 8.0 stock to a 100 mL graduated cylinder and add water to a final volume 100 mL.

D. 200 mM 1,4-Dithiothreitol (DTT)
(This solution must be prepared fresh for each process replicate.)

1. Transfer the pre-weighed 7.7 mg aliquot of DTT to a larger 1.5 mL Eppendorf tube by adding 100 ul of water, wait until the DTT dissolves, pipette this liquid to the new tube, and then add another 150 μL of water resulting in a final concentration of 200 mM (don't transfer the dry solid).

E. 400 mM Iodoacetamide (IAM)
(Prepare immediately before use and keep out of the light)

1. To one 56 mg vial of iodoacetamide, add 757 μL of water for a final concentration of 400 mM.

Document S3: Study 9.1 & 9.2 (Phase II & III) – summary of instrument operating parameters.

Mass Spectrometer Operating Parameters: General instrument operating parameters were kept as uniform as possible across the different MS platforms, except where noted in the individual sections below (additional details found in the Supplementary Documents 1 and 2 and Supplementary Tables 2 and 3). All MRM transitions are listed in **Supplementary Table 2** for each instrument platform**.** A total of 750 MRM transitions were monitored for Phase II and 1095 for Phase III. Q1 and Q3 were set to unit resolution for MRM acquisition. Data were collected in retention time scheduled MRM mode and the total cycle time for each instrument was set to about 1 second. The specific parameters used for each instrument platform are detailed below.

ABI 4000 QTRAP / QTRAP 5500 Mass Spectrometer: Nine 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometers (AB Sciex, Foster City, CA) located at different sites were used to acquire MRM-MS data for this study. Ion spray voltages of 2200 ± 200 V, curtain gas 20, nebulizer gas (GS1) 5 ± 2, and interface heater temperature (IHT) 150°C were used. Declustering potential (DP), and collision energy (CE) were calculated in Skyline using the following regression equations: DP = 0.0729**m/z* + 31.117 and CE = 0.0431**m/z* + 4.7556 (from personal communication with Dr. Jeffrey Whiteaker). Interscan delays were set to 5 msec. The QTRAP 5500 instrument was operated in a similar manner. See Supplementary Table S2a for MRM transition used. The following CE linear equations were used: CE=(0.036**m/z* + 8.857) for z=2 and CE=(0.0544**m/z* - 2.41) for z=3. For the QTRAP 5500 an interscan delay of 3 msec was used for all transitions. Analyst version 1.5 was used as operating software.

TSQ Vantage Mass Spectrometer: The TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA) was operated with a spray voltage of 1300 V, capillary temperature of 210 ºC, Q2 Argon gas pressure of 1.5 mTorr and scan width of 0.005 *m/z*.. TSQ Vantage 2.0.0 and Xcalibur 2.0.7 SP1 operating software were used. See Supplementary Table S2b for MRM transition used.

Waters Xevo TQ Mass Spectrometer: Two Waters Xevo TQ and one Xevo TQS triple quadrupole mass spectrometers (Milford, MA) were operated with capillary voltage was set to 2.2 kV, the collision gas to 4 mL/min, and the Cone Voltage to 37 V. MS Inter-scan time and the Inter-channel Delay were both set to 3.5 msec. The CE for Waters instruments was calculated

within Skyline using the linear equation CE=0.034*m/z + 3.314. MassLynx version 4.1 SCM725 was used. See Supplementary Table S2c for MRM transition used.

Agilent ChipCube Mass Spectrometer: One Agilent 6410, one 6460, and one 6490 triple quadrupole mass spectrometers (Santa Clara, CA) coupled to ChipCube interfaces were used to acquire MRM data. The CE for Agilent instruments was calculated in Skyline using the linear equation CE=0.036*m/z – 4.8. Source settings for the 6410 and 6490 were as follows: ESI voltage was 1750 V with a gas flow of 2.5 L/min, source temperature of 325 ˚C and fragmentor voltage of 125 V. For the 6490, ESI voltage was 1900 V with gas temperature of 150MassHunter version 4.01 was used. See Supplementary Table S2d for MRM transition used.

Supplemental Table SA-E: MRM-MS transition lists for each vendor platform (displayed for light transitions)

Supplemental Table S2A: AB Sciex (4000 QTRAP) instrument transition lists

Supplemental Table S2B: Thermo instrument transition lists

Supplemental Table S2C: Waters instrument transition lists

Supplemental Table S2D: Agilent instrument transition lists

Supplemental Table S2E: AB Sciex (QTRAP 5500) instrument transition lists

Supplemental Table S2A: MRM-MS transition list for each vendor platform, AB Sciex (4000 QTRAP)

S2A: AB Sciex, 4000 QTRAP transition lists (displayed for light transitions)

Peptide Sequence	Protein Name	Precursor Mz	Precursor Charge	Product Mz	Product Charge	Fragment Ion	Collision Energy
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	888.47	1	y8	30.0
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	817.43	1	y7	30.0
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	659.36	1	y5	30.0
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	758.42	1	y8	25.9
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	644.37	1	y7	25.9
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	474.27	1	y5	25.9
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	674.38	1	y6	21.9
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	472.32	1	y4	21.9
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	359.24	1	y3	21.9
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	1112.64	1	y10	42.9
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	999.56	1	y9	42.9
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	886.47	1	y8	42.9
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	823.39	1	y7	25.7
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	710.31	1	y6	25.7
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	563.24	1	y5	25.7
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	724.33	1	y6	21.7
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	561.26	1	y5	21.7
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	432.22	1	y4	21.7
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1178.65	1	y10	40.0
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1064.61	1	y9	40.0
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	965.54	1	y8	40.0
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	987.61	1	y9	35.3
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	916.57	1	y8	35.3
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	702.44	1	y6	35.3
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	1101.62	1	y10	25.5
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	853.45	2	y15	25.5
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	736.40	2	y13	25.5
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	1064.50	1	y9	31.7
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	993.46	1	y8	31.7
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	865.41	1	y7	31.7
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	746.42	1	y8	23.1
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	647.35	1	y7	23.1
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	447.23	1	y5	23.1
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	708.40	1	y6	24.6
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	621.37	1	y5	24.6
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	522.30	1	y4	24.6
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	964.51	1	y8	35.1
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	801.45	1	y7	35.1
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	688.36	1	y6	35.1
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	1004.50	1	y9	29.2
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	846.43	1	y7	29.2
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	731.40	1	y6	29.2
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	1131.62	1	y10	43.1
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	801.48	1	y7	43.1
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	446.27	1	y4	43.1
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	617.30	1	y6	21.2
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	489.24	1	y5	21.2
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	418.20	1	y4	21.2
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	828.52	1	y7	25.5
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	715.43	1	y6	25.5
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	600.41	1	y5	25.5
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	963.47	1	y9	26.9
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	805.41	1	y7	26.9
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	690.38	1	y6	26.9
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	939.48	1	y9	30.4
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	632.36	1	y6	30.4
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	533.29	1	y5	30.4
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	772.43	1	y7	24.6
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	673.36	1	y6	24.6
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	459.27	1	y4	24.6
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	751.33	1	y6	24.0
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	636.30	1	y5	24.0
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	522.26	1	y4	24.0
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	805.38	1	y7	23.6
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	677.32	1	y6	23.6
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	576.27	1	y5	23.6
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	1113.52	1	y10	41.6
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	955.45	1	y8	41.6
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	592.31	1	y5	41.6
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	1153.53	1	y10	36.0
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	893.37	1	y8	36.0
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	778.35	1	y7	36.0
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	1034.62	1	y9	30.5
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	820.49	1	y7	30.5
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	721.42	1	y6	30.5
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	845.49	1	y7	27.0
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	732.41	1	y6	27.0
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	633.34	1	y5	27.0
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	681.33	1	y6	23.1
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	594.30	1	y5	23.1
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	409.22	1	y3	23.1
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	642.34	1	y5	23.4
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	529.26	1	y4	23.4
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	369.22	1	y3	23.4
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	1024.53	1	y9	32.3
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	877.46	1	y8	32.3
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	664.35	1	y6	32.3
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	619.32	1	y5	25.3
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	310.16	2	y5	25.3
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	306.14	1	b3	25.3
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	649.32	1	y5	19.3
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	512.26	1	y4	19.3
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	467.74	2	y7	19.3
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	884.40	1	y6	21.1
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	755.36	1	y5	21.1
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	626.32	1	y4	21.1
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	591.31	1	y5	21.4
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	478.23	1	y4	21.4
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	903.47	1	y8	28.9
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	660.38	1	y6	28.9
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	589.35	1	y5	28.9
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	742.41	1	y8	22.6
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	629.33	1	y7	22.6
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	572.30	1	y6	22.6
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	872.47	1	y8	25.5
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	801.44	1	y7	25.5
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	842.93	2	y15	25.5
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	646.38	1	y6	21.9
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	547.31	1	y5	21.9
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	418.27	1	y4	21.9
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	834.43	1	y8	26.4
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	660.37	1	y6	26.4
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	504.28	1	y4	26.4
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	704.32	1	y6	19.5
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	589.29	1	y5	19.5
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	460.25	1	y4	19.5
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	710.40	1	y5	25.0
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	597.31	1	y4	25.0
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	450.25	1	y3	25.0
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	901.49	1	y7	27.6
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	754.42	1	y6	27.6
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	641.33	1	y5	27.6
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	938.42	1	y8	25.7
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	851.39	1	y7	25.7
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	637.26	1	y5	25.7
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	717.45	1	y7	21.6
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	646.41	1	y6	21.6
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	547.34	1	y5	21.6
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	847.41	1	y7	26.4
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	776.37	1	y6	26.4
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	590.29	1	y5	26.4
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	670.34	1	y5	22.5
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	533.28	1	y4	22.5
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	642.82	2	y10	22.5
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	720.37	1	y6	24.1
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	607.29	1	y5	24.1
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	508.22	1	y4	24.1
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	643.41	1	y6	20.8
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	544.35	1	y5	20.8
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	431.26	1	y4	20.8
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	855.39	1	y7	23.4
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	758.34	1	y6	23.4
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	428.20	2	y7	23.4
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	818.51	1	y8	28.2
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	761.49	1	y7	28.2
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	648.41	1	y6	28.2
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1103.61	1	y10	33.4
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1002.56	1	y9	33.4
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	887.53	1	y8	33.4
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	788.43	1	y8	23.1
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	717.39	1	y7	23.1
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	517.27	1	y5	23.1
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	959.50	1	y9	31.2
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	872.47	1	y8	31.2
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	662.34	1	y6	31.2
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	913.51	1	y8	29.3
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	784.47	1	y7	29.3
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	656.41	1	y6	29.3
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	692.40	1	y6	20.3
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	545.33	1	y5	20.3
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	432.25	1	y4	20.3
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	708.37	1	y6	22.1
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	579.32	1	y5	22.1
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	409.22	1	y3	22.1
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	910.45	1	y8	24.9
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	823.42	1	y7	24.9
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	694.38	1	y6	24.9
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	806.44	1	y8	23.7
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	735.40	1	y7	23.7
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	636.34	1	y6	23.7
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	1027.52	1	y9	23.9
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	914.43	1	y8	23.9
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	785.39	1	y7	23.9
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	1000.47	1	y8	30.3
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	872.41	1	y7	30.3
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	801.38	1	y6	30.3
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	1028.54	1	y9	22.2
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	676.38	2	y12	22.2
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	627.86	2	y11	22.2
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	770.42	1	y7	30.7
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	656.37	1	y6	30.7
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	541.35	1	y5	30.7
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	865.44	1	y8	24.6
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	668.84	2	y12	24.6
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	554.29	2	y10	24.6
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	763.40	1	y7	24.4
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	666.35	1	y6	24.4
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	579.31	1	y5	24.4
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	736.29	1	y6	23.9
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	576.26	1	y5	23.9
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	448.20	1	y4	23.9
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	1108.52	1	y9	24.7
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	894.42	1	y7	24.7
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	747.35	1	y6	24.7
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	1002.53	1	y8	26.0
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	622.83	2	y10	26.0

SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	391.19	1	y3	21.4	ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	558.31	2	y9	26.0
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	801.45	1	y7	21.8	EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	642.43	1	y6	21.4
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	730.41	1	y6	21.8	EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	543.36	1	y5	21.4
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	617.33	1	y5	21.8	EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	430.28	1	y4	21.4
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	795.42	1	y7	24.2	IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	930.48	1	y8	28.8
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	682.34	1	y6	24.2	IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	817.39	1	y7	28.8
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	611.30	1	y5	24.2	IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	702.37	1	y6	28.8
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	870.44	1	y8	28.4	ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	889.46	1	y8	24.3
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	769.39	1	y7	28.4	ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	776.38	1	y7	24.3
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	698.35	1	y6	28.4	ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	662.34	1	y6	24.3
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	670.29	1	y6	18.9	EQANAVSEAVVSSVNTVA	sp|O76070|SYUG_HUMAN	668.68	3	1005.56	1	y10	27.8
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	441.73	2	y8	18.9	EQANAVSEAVVSSVNTVA	sp|O76070|SYUG_HUMAN	668.68	3	906.49	1	y9	27.8
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	385.18	2	y7	18.9	EQANAVSEAVVSSVNTVA	sp|O76070|SYUG_HUMAN	668.68	3	819.46	1	y8	27.8
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	914.49	1	y10	29.7	EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	602.35	1	y6	21.1
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	843.46	1	y9	29.7	EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	503.28	1	y5	21.1
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	746.40	1	y8	29.7	EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	446.26	1	y4	21.1
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	746.38	1	y7	22.7	ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	1047.57	1	y10	35.4
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	588.31	1	y5	22.7	ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	948.50	1	y9	35.4
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	475.23	1	y4	22.7	ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	820.44	1	y8	35.4
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	930.49	1	y9	36.7	TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	717.43	1	y7	24.4
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	731.39	1	y7	36.7	TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	618.36	1	y6	24.4
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	531.28	1	y5	36.7	TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	517.31	1	y5	24.4
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1154.58	1	y10	41.6	HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	676.29	1	y6	22.2
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1083.55	1	y9	41.6	HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	476.21	1	y4	22.2
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	913.44	1	y7	41.6	HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	361.18	1	y3	22.2
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	931.42	1	y8	26.0	LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	974.52	1	y8	29.6
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	834.36	1	y7	26.0	LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	861.43	1	y7	29.6
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	519.26	1	y4	26.0	LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	748.35	1	y6	29.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	606.31	1	y6	20.2	SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	995.47	1	y8	39.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	639.31	1	b6	20.2	SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	866.43	1	y7	39.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	710.35	1	b7	20.2	SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	751.40	1	y6	39.6
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1187.58	1	y10	34.9	QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	1060.55	1	y9	32.9
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1100.55	1	y9	34.9	QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	687.44	1	y6	32.9
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	971.50	1	y8	34.9	QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	574.36	1	y5	32.9
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	645.36	1	y5	19.9	YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	868.44	1	y7	25.2
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	516.31	1	y4	19.9	YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	755.36	1	y6	25.2
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	387.27	1	y3	19.9	YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	627.30	1	y5	25.2
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	1031.50	1	y10	25.0	WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	823.43	1	y7	24.5
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	889.43	1	y8	25.0	WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	736.40	1	y6	24.5
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	632.32	1	y5	25.0	WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	552.28	1	y4	24.5
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	899.49	1	y9	27.8	LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	889.48	1	y8	26.6
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	614.36	1	y6	27.8	LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	792.43	1	y7	26.6
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	543.32	1	y5	27.8	LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	629.36	1	y6	26.6
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	994.53	1	y8	26.6	DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	746.42	1	y8	25.1
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	831.47	1	y7	26.6	DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	675.38	1	y7	25.1
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	703.41	1	y6	26.6	DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	574.33	1	y6	25.1
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1171.60	1	y10	39.5	GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	1096.53	1	y9	36.4
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1058.52	1	y9	39.5	GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	949.46	1	y8	36.4
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	945.43	1	y8	39.5	GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	852.41	1	y7	36.4
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1077.52	1	y9	45.2	DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	515.32	1	y5	20.7
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	948.48	1	y8	45.2	DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	345.21	1	y3	20.7
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	847.43	1	y7	45.2	DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	701.30	1	b6	20.7
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	879.51	1	y7	33.9	GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	836.43	1	y7	26.9
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	780.44	1	y6	33.9	GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	650.35	1	y6	26.9
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	681.37	1	y5	33.9	GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	467.25	2	y8	26.9
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	818.48	1	y7	28.2	DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	775.40	1	y7	24.4
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	705.39	1	y6	28.2	DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	612.34	1	y6	24.4
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	542.33	1	y5	28.2	DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	341.22	1	y3	24.4
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	1048.50	1	y8	35.5	DWRPAITIK	sp|P63279|UBC9_HUMAN	550.32	2	492.80	2	y8	27.1
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	885.44	1	y7	35.5	DWRPAITIK	sp|P63279|UBC9_HUMAN	550.32	2	840.44	1	b7	27.1
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	738.37	1	y6	35.5	DWRPAITIK	sp|P63279|UBC9_HUMAN	550.32	2	953.52	1	b8	27.1
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	701.33	1	y5	21.5	AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	1087.50	1	y9	38.2
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	586.30	1	y4	21.5	AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	959.44	1	y8	38.2
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	439.23	1	y3	21.5	AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	858.39	1	y7	38.2
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	703.39	1	y6	19.0	INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	720.39	1	y7	21.6
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	632.35	1	y5	19.0	INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	643.82	2	y12	21.6
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	446.27	1	y4	19.0	INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	586.80	2	y11	21.6
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	628.28	1	y5	25.6	LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	716.38	1	y6	20.2
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	470.22	2	y8	25.6	LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	579.79	2	y10	20.2
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	753.40	1	b8	25.6	LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	506.26	2	y9	20.2
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	674.37	1	y6	19.2	YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	817.36	1	y7	20.8
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	545.33	1	y5	19.2	YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	523.74	2	y10	20.8
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	416.29	1	y4	19.2	YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	488.22	2	y9	20.8
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	802.42	1	y6	22.7	HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	589.35	1	y5	16.5
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	689.34	1	y5	22.7	HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	532.32	1	y4	16.5
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	590.27	1	y4	22.7	HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	385.26	1	y3	16.5
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	773.39	1	y6	18.4	LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	943.51	1	y9	32.0
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	658.37	1	y5	18.4	LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	846.46	1	y8	32.0
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	559.30	1	y4	18.4	LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	775.42	1	y7	32.0
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	685.40	1	y6	23.2	IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	964.42	1	y8	26.5
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	586.33	1	y5	23.2	IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	865.35	1	y7	26.5
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	485.28	1	y4	23.2	IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	808.33	1	y6	26.5
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	924.43	1	y8	27.0	SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	974.51	1	y10	22.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	853.39	1	y7	27.0	SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	790.38	1	y8	22.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	738.37	1	y6	27.0	SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	703.35	1	y7	22.4
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1107.57	1	y11	37.2	GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	916.48	1	y8	28.2
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1020.54	1	y10	37.2	GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	829.45	1	y7	28.2
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	949.50	1	y9	37.2	GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	716.36	1	y6	28.2
								ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	912.47	1	y8	27.9
								ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	797.44	1	y7	27.9
								ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	696.39	1	y6	27.9

Supplemental Table S2B: MRM-MS transition list for each vendor platform, Thermo

S2B: Thermo instrument transition lists (displayed for light transitions)

Peptide Sequence	Protein Name	Precursor Mz	Precursor Charge	Product Mz	Product Charge	Fragment Ion	Collision Energy
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	888.47	1	y8	20.9
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	817.43	1	y7	20.9
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	659.36	1	y5	20.9
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	857.48	1	y9	18.8
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	758.42	1	y8	18.8
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	644.37	1	y7	18.8
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	674.38	1	y6	16.7
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	587.35	1	y5	16.7
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	472.32	1	y4	16.7
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	1112.64	1	y10	27.7
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	999.56	1	y9	27.7
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	886.47	1	y8	27.7
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	823.39	1	y7	18.7
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	710.31	1	y6	18.7
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	563.24	1	y5	18.7
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	724.33	1	y6	16.5
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	561.26	1	y5	16.5
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	477.23	1	b4	16.5
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1178.65	1	y10	26.2
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	965.54	1	y8	26.2
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	818.47	1	y7	26.2
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	916.57	1	y8	23.7
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	815.52	1	y7	23.7
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	702.44	1	y6	23.7
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	815.45	1	y7	24.8
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	817.94	2	y14	24.8
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	736.40	2	y13	24.8
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	1064.50	1	y9	21.9
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	865.41	1	y7	21.9
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	718.34	1	y6	21.9
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	746.42	1	y8	17.3
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	647.35	1	y7	17.3
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	560.32	1	y6	17.3
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	846.94	2	1120.64	1	y10	28.3
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	846.94	2	1005.61	1	y9	28.3
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	846.94	2	934.57	1	y8	28.3
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	1135.57	1	y10	23.6
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	964.51	1	y8	23.6
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	801.45	1	y7	23.6
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	846.43	1	y7	20.5
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	731.40	1	y6	20.5
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	616.38	1	y5	20.5
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	1131.62	1	y10	27.8
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	1074.60	1	y9	27.8
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	914.57	1	y8	27.8
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	780.36	1	y7	16.3
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	617.30	1	y6	16.3
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	489.24	1	y5	16.3
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	828.52	1	y7	18.6
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	715.43	1	y6	18.6
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	600.41	1	y5	18.6
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	963.47	1	y9	19.3
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	805.41	1	y7	19.3
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	690.38	1	y6	19.3
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	939.48	1	y9	21.2
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	852.45	1	y8	21.2
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	632.36	1	y6	21.2
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	845.92	2	1084.61	1	y10	28.3
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	845.92	2	956.55	1	y9	28.3
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	845.92	2	885.52	1	y8	28.3
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	751.33	1	y6	17.8
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	636.30	1	y5	17.8
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	522.26	1	y4	17.8
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	805.38	1	y7	17.6
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	677.32	1	y6	17.6
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	576.27	1	y5	17.6
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	1113.52	1	y10	27.0
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	1026.49	1	y9	27.0
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	955.45	1	y8	27.0
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	1006.46	1	y9	24.1
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	893.37	1	y8	24.1
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	778.35	1	y7	24.1
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	1034.62	1	y9	21.2
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	820.49	1	y7	21.2
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	721.42	1	y6	21.2
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	845.49	1	y7	19.4
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	732.41	1	y6	19.4
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	633.34	1	y5	19.4
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	794.42	1	y7	17.3
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	681.33	1	y6	17.3
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	594.30	1	y5	17.3
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	786.91	2	1054.57	1	y9	26.5
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	786.91	2	941.49	1	y8	26.5
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	786.91	2	812.44	1	y7	26.5
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	877.46	1	y8	22.1
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	763.42	1	y7	22.1
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	664.35	1	y6	22.1
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	833.45	1	y7	18.5
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	732.40	1	y6	18.5
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	619.32	1	y5	18.5
IQVWHAEHR	sp|P15311|EZRI_HUMAN	588.31	2	934.46	1	y7	20.6
IQVWHAEHR	sp|P15311|EZRI_HUMAN	588.31	2	835.40	1	y6	20.6
IQVWHAEHR	sp|P15311|EZRI_HUMAN	588.31	2	649.32	1	y5	20.6
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	678.79	2	983.47	1	y7	23.3
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	678.79	2	884.40	1	y6	23.3
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	678.79	2	755.36	1	y5	23.3
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	754.37	1	y6	16.4
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	591.31	1	y5	16.4
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	903.47	1	y8	20.4
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	660.38	1	y6	20.4
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	589.35	1	y5	20.4
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	742.41	1	y8	17.0
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	629.33	1	y7	17.0
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	572.30	1	y6	17.0
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	872.47	1	y8	24.9
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	801.44	1	y7	24.9
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	842.93	2	y15	24.9
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	745.45	1	y7	16.7
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	646.38	1	y6	16.7
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	547.31	1	y5	16.7
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	834.43	1	y8	19.1
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	747.40	1	y7	19.1
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	660.37	1	y6	19.1
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	704.32	1	y6	15.4
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	589.29	1	y5	15.4
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	460.25	1	y4	15.4
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	823.48	1	y6	18.3
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	710.40	1	y5	18.3
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	597.31	1	y4	18.3
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	901.49	1	y7	19.7
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	754.42	1	y6	19.7
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	641.33	1	y5	19.7
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	938.42	1	y8	18.7
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	851.39	1	y7	18.7
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	637.26	1	y5	18.7
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	717.45	1	y7	16.5
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	646.41	1	y6	16.5
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	547.34	1	y5	16.5
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	847.41	1	y7	19.1
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	776.37	1	y6	19.1
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	590.29	1	y5	19.1
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	783.43	1	y6	21.1
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	670.34	1	y5	21.1
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	533.28	1	y4	21.1
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	720.37	1	y6	23.2
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	607.29	1	y5	23.2
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	827.34	1	b8	23.2
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	790.48	1	y7	16.1
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	643.41	1	y6	16.1
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	544.35	1	y5	16.1
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	855.39	1	y7	17.4
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	758.34	1	y6	17.4
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	643.31	1	y5	17.4
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	648.41	1	y6	20.0
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	588.28	1	b6	20.0
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	645.30	1	b7	20.0
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1103.61	1	y10	22.7
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	887.53	1	y8	22.7
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	773.49	1	y7	22.7
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	788.43	1	y8	17.3
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	717.39	1	y7	17.3
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	517.27	1	y5	17.3
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	959.50	1	y9	21.6
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	872.47	1	y8	21.6
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	662.34	1	y6	21.6
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	913.51	1	y8	20.6
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	784.47	1	y7	20.6
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	656.41	1	y6	20.6
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	692.40	1	y6	15.9
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	545.33	1	y5	15.9
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	346.70	2	y6	15.9
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	708.37	1	y6	16.8
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	579.32	1	y5	16.8
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	522.30	1	y4	16.8
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	910.45	1	y8	18.3
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	823.42	1	y7	18.3
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	694.38	1	y6	18.3
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	806.44	1	y8	17.6
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	735.40	1	y7	17.6
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	636.34	1	y6	17.6
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	1027.52	1	y9	22.9
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	914.43	1	y8	22.9
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	785.39	1	y7	22.9
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	1000.47	1	y8	21.1
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	872.41	1	y7	21.1
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	801.38	1	y6	21.1
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	676.38	2	y12	20.9
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	571.31	2	y10	20.9
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	514.77	2	y9	20.9
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	883.50	1	y8	21.3
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	770.42	1	y7	21.3
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	656.37	1	y6	21.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	846.45	2	1208.62	1	y11	28.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	846.45	2	978.53	1	y9	28.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	846.45	2	865.44	1	y8	28.3
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	763.40	1	y7	18.0
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	666.35	1	y6	18.0
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	579.31	1	y5	18.0
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	823.33	1	y7	17.7
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	736.29	1	y6	17.7
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	576.26	1	y5	17.7
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	853.94	2	1108.52	1	y9	28.5
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	853.94	2	993.49	1	y8	28.5
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	853.94	2	894.42	1	y7	28.5
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	1002.53	1	y8	25.5
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	736.87	2	y12	25.5

SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	478.23	1	y4	16.4
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	801.45	1	y7	16.6
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	730.41	1	y6	16.6
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	617.33	1	y5	16.6
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	826.41	2	1066.54	1	y10	27.7
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	826.41	2	995.50	1	y9	27.7
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	826.41	2	924.47	1	y8	27.7
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	870.44	1	y8	20.1
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	769.39	1	y7	20.1
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	698.35	1	y6	20.1
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	571.80	2	769.36	1	y7	20.1
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	571.80	2	670.29	1	y6	20.1
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	571.80	2	599.25	1	y5	20.1
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	914.49	1	y10	20.8
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	843.46	1	y9	20.8
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	746.40	1	y8	20.8
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	746.38	1	y7	17.1
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	588.31	1	y5	17.1
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	475.23	1	y4	17.1
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	930.49	1	y9	24.5
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	859.45	1	y8	24.5
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	731.39	1	y7	24.5
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1154.58	1	y10	27.0
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1083.55	1	y9	27.0
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	913.44	1	y7	27.0
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	931.42	1	y8	18.8
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	834.36	1	y7	18.8
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	670.30	1	b6	18.8
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	719.39	1	y7	15.8
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	606.31	1	y6	15.8
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	639.31	1	b6	15.8
WSEPNEEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1187.58	1	y10	23.5
WSEPNEEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1100.55	1	y9	23.5
WSEPNEEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	971.50	1	y8	23.5
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	645.36	1	y5	15.6
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	516.31	1	y4	15.6
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	459.21	1	b4	15.6
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	864.43	2	1031.50	1	y10	28.8
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	864.43	2	960.46	1	y9	28.8
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	864.43	2	889.43	1	y8	28.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	899.49	1	y9	19.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	713.43	1	y7	19.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	614.36	1	y6	19.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	942.48	2	1208.66	1	y10	31.2
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	942.48	2	1107.62	1	y9	31.2
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	942.48	2	994.53	1	y8	31.2
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1058.52	1	y9	25.9
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	945.43	1	y8	25.9
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	782.37	1	y7	25.9
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1277.64	1	y11	28.9
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1077.52	1	y9	28.9
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	948.48	1	y8	28.9
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	980.56	1	y8	23.0
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	780.44	1	y6	23.0
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	681.37	1	y5	23.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	978.51	1	y8	20.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	818.48	1	y7	20.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	705.39	1	y6	20.0
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	1048.50	1	y8	23.9
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	885.44	1	y7	23.9
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	738.37	1	y6	23.9
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	814.41	1	y6	16.4
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	701.33	1	y5	16.4
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	586.30	1	y4	16.4
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	703.39	1	y6	15.2
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	632.35	1	y5	15.2
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	446.27	1	y4	15.2
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	939.42	1	y8	25.0
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	628.28	1	y5	25.0
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	753.40	1	b8	25.0
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	674.37	1	y6	15.2
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	545.33	1	y5	15.2
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	416.29	1	y4	15.2
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	802.42	1	y6	21.4
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	689.34	1	y5	21.4
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	590.27	1	y4	21.4
FGNDVQHFK	sp|P62993|GRB2_HUMAN	546.27	2	944.46	1	y8	19.3
FGNDVQHFK	sp|P62993|GRB2_HUMAN	546.27	2	887.44	1	y7	19.3
FGNDVQHFK	sp|P62993|GRB2_HUMAN	546.27	2	658.37	1	y5	19.3
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	685.40	1	y6	17.4
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	586.33	1	y5	17.4
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	485.28	1	y4	17.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	924.43	1	y8	19.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	853.39	1	y7	19.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	738.37	1	y6	19.4
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1107.57	1	y11	24.7
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1020.54	1	y10	24.7
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	949.50	1	y9	24.7

ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	679.85	2	y11	25.5
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	642.43	1	y6	16.4
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	543.36	1	y5	16.4
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	470.26	1	b4	16.4
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	930.48	1	y8	20.3
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	817.39	1	y7	20.3
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	702.37	1	y6	20.3
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	889.46	1	y8	18.0
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	776.38	1	y7	18.0
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	662.34	1	y6	18.0
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	1005.56	1	y10	27.7
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	906.49	1	y9	27.7
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	819.46	1	y8	27.7
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	602.35	1	y6	16.2
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	503.28	1	y5	16.2
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	446.26	1	y4	16.2
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	1047.57	1	y10	23.8
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	948.50	1	y9	23.8
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	820.44	1	y8	23.8
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	837.44	2	1144.63	1	y11	28.0
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	837.44	2	1015.59	1	y10	28.0
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	837.44	2	901.55	1	y9	28.0
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	789.37	1	y7	16.8
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	676.29	1	y6	16.8
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	566.26	1	b5	16.8
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	974.52	1	y8	20.7
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	861.43	1	y7	20.7
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	748.35	1	y6	20.7
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	1108.55	1	y9	26.0
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	995.47	1	y8	26.0
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	866.43	1	y7	26.0
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	1060.55	1	y9	22.4
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	931.51	1	y8	22.4
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	687.44	1	y6	22.4
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	868.44	1	y7	18.4
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	755.36	1	y6	18.4
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	627.30	1	y5	18.4
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	823.43	1	y7	18.1
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	736.40	1	y6	18.1
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	665.36	1	y5	18.1
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	941.98	2	1293.65	1	y12	31.2
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	941.98	2	1196.59	1	y11	31.2
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	941.98	2	1109.56	1	y10	31.2
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	746.42	1	y8	18.4
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	675.38	1	y7	18.4
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	574.33	1	y6	18.4
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	1096.53	1	y9	24.3
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	949.46	1	y8	24.3
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	723.37	1	y6	24.3
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	775.40	1	y7	18.0
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	612.34	1	y6	18.0
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	515.28	1	y5	18.0
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	933.48	1	y8	19.3
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	836.43	1	y7	19.3
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	650.35	1	y6	19.3
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	399.76	2	y7	16.2
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	458.21	1	b3	16.2
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	626.30	1	b5	16.2
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	515.32	1	y5	19.0
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	554.24	1	b5	19.0
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	701.30	1	b6	19.0
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	1087.50	1	y9	25.3
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	959.44	1	y8	25.3
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	858.39	1	y7	25.3
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	720.39	1	y7	20.0
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	643.82	2	y12	20.0
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	586.80	2	y11	20.0
LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	716.38	1	y6	18.4
LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	579.79	2	y10	18.4
LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	506.26	2	y9	18.4
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	817.36	1	y7	19.1
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	730.33	1	y6	19.1
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	629.28	1	y5	19.1
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	589.35	1	y5	13.8
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	532.32	1	y4	13.8
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	385.26	1	y3	13.8
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	424.89	3	580.30	2	y11	18.4
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	424.89	3	498.26	1	b5	18.4
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	424.89	3	627.30	1	b6	18.4
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	865.35	1	y7	19.1
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	808.33	1	y6	19.1
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	751.31	1	y5	19.1
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	974.51	1	y10	21.0
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	790.38	1	y8	21.0
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	703.35	1	y7	21.0
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	916.48	1	y8	20.0
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	716.36	1	y6	20.0
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	569.29	1	y5	20.0
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	797.44	1	y7	19.8
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	696.39	1	y6	19.8
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	609.36	1	y5	19.8

Supplemental Table S2C: MRM-MS transition list for each vendor platform, Waters

S2C: Waters instrument transition lists (displayed for light transitions)

Peptide Sequence	Protein Name	Precursor Mz	Precursor Charge	Product Mz	Product Charge	Fragment Ion	Collision Energy
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	888.47	1	y8	21.2
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	817.43	1	y7	21.2
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	659.36	1	y5	21.2
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	758.42	1	y8	18.5
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	644.37	1	y7	18.5
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	474.27	1	y5	18.5
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	674.38	1	y6	15.9
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	472.32	1	y4	15.9
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	359.24	1	y3	15.9
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	1112.64	1	y10	29.5
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	999.56	1	y9	29.5
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	541.30	1	b6	29.5
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	823.39	1	y7	18.4
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	710.31	1	y6	18.4
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	563.24	1	y5	18.4
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	837.41	1	y7	15.8
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	561.26	1	y5	15.8
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	432.22	1	y4	15.8
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1178.65	1	y10	27.6
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	965.54	1	y8	27.6
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	818.47	1	y7	27.6
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	916.57	1	y8	24.6
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	815.52	1	y7	24.6
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	702.44	1	y6	24.6
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	888.97	2	1101.62	1	y10	31.8
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	888.97	2	1000.57	1	y9	31.8
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	888.97	2	815.45	1	y7	31.8
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	1064.50	1	y9	22.3
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	865.41	1	y7	22.3
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	718.34	1	y6	22.3
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	746.42	1	y8	16.7
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	647.35	1	y7	16.7
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	447.23	1	y5	16.7
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	708.40	1	y6	19.0
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	621.37	1	y5	19.0
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	522.30	1	y4	19.0
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	964.51	1	y8	24.5
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	801.45	1	y7	24.5
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	688.36	1	y6	24.5
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	1004.50	1	y9	20.7
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	846.43	1	y7	20.7
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	731.40	1	y6	20.7
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	1131.62	1	y10	29.7
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	914.57	1	y8	29.7
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	801.48	1	y7	29.7
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	780.36	1	y7	15.5
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	617.30	1	y6	15.5
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	489.24	1	y5	15.5
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	828.52	1	y7	18.3
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	715.43	1	y6	18.3
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	487.32	1	y4	18.3
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	963.47	1	y9	19.1
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	805.41	1	y7	19.1
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	690.38	1	y6	19.1
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	939.48	1	y9	21.4
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	632.36	1	y6	21.4
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	533.29	1	y5	21.4
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	845.92	2	956.55	1	y9	30.2
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	845.92	2	772.43	1	y7	30.2
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	845.92	2	673.36	1	y6	30.2
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	751.33	1	y6	17.3
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	636.30	1	y5	17.3
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	522.26	1	y4	17.3
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	1113.52	1	y10	28.7
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	955.45	1	y8	28.7
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	592.31	1	y5	28.7
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	1153.53	1	y10	25.0
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	893.37	1	y8	25.0
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	778.35	1	y7	25.0
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	1034.62	1	y9	21.5
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	820.49	1	y7	21.5
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	721.42	1	y6	21.5
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	845.49	1	y7	19.2
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	732.41	1	y6	19.2
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	633.34	1	y5	19.2
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	681.33	1	y6	16.7
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	594.30	1	y5	16.7
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	409.22	1	y3	16.7
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	786.91	2	941.49	1	y8	28.0
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	786.91	2	812.44	1	y7	28.0
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	786.91	2	642.34	1	y5	28.0
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	877.46	1	y8	22.7
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	763.42	1	y7	22.7
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	664.35	1	y6	22.7
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	833.45	1	y7	18.1
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	732.40	1	y6	18.1
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	619.32	1	y5	18.1
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	512.26	1	y4	12.8
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	467.74	2	y7	12.8
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	418.20	2	y6	12.8
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	678.79	2	884.40	1	y6	24.0
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	678.79	2	755.36	1	y5	24.0
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	678.79	2	626.32	1	y4	24.0
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	754.37	1	y6	15.6
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	591.31	1	y5	15.6
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	478.23	1	y4	15.6
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	730.41	1	y6	15.9
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	617.33	1	y5	15.9
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	903.47	1	y8	20.5
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	660.38	1	y6	20.5
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	589.35	1	y5	20.5
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	742.41	1	y8	16.4
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	629.33	1	y7	16.4
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	572.30	1	y6	16.4
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	1156.60	1	y10	20.1
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	1019.54	1	y9	20.1
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	872.47	1	y8	20.1
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	646.38	1	y6	15.9
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	547.31	1	y5	15.9
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	418.27	1	y4	15.9
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	834.43	1	y8	18.9
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	660.37	1	y6	18.9
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	504.28	1	y4	18.9
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	589.29	1	y5	14.4
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	460.25	1	y4	14.4
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	347.17	1	y3	14.4
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	710.40	1	y5	17.9
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	597.31	1	y4	17.9
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	450.25	1	y3	17.9
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	901.49	1	y7	19.6
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	754.42	1	y6	19.6
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	641.33	1	y5	19.6
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	938.42	1	y8	18.4
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	851.39	1	y7	18.4
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	637.26	1	y5	18.4
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	717.45	1	y7	15.7
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	646.41	1	y6	15.7
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	547.34	1	y5	15.7
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	847.41	1	y7	18.9
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	776.37	1	y6	18.9
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	590.29	1	y5	18.9
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	743.85	2	943.46	1	y7	26.5
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	743.85	2	670.34	1	y5	26.5
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	743.85	2	533.28	1	y4	26.5
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	720.37	1	y6	18.5
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	607.29	1	y5	18.5
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	508.22	1	y4	18.5
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	643.41	1	y6	15.2
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	544.35	1	y5	15.2
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	431.26	1	y4	15.2
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	855.39	1	y7	16.9
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	758.34	1	y6	16.9
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	643.31	1	y5	16.9
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	818.51	1	y8	20.0
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	648.41	1	y6	20.0
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	549.34	1	y5	20.0
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1103.61	1	y10	23.4
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	773.49	1	y7	23.4
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	660.40	1	y6	23.4
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	788.43	1	y8	16.7
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	717.39	1	y7	16.7
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	517.27	1	y5	16.7
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	959.50	1	y9	22.0
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	872.47	1	y8	22.0
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	662.34	1	y6	22.0
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	784.47	1	y7	20.7
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	656.41	1	y6	20.7
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	446.27	1	y4	20.7
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	692.40	1	y6	14.9
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	403.24	2	y7	14.9
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	346.70	2	y6	14.9
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	708.37	1	y6	16.1
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	579.32	1	y5	16.1
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	409.22	1	y3	16.1
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	910.45	1	y8	17.9
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	823.42	1	y7	17.9
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	694.38	1	y6	17.9
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	806.44	1	y8	17.1
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	735.40	1	y7	17.1
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	636.34	1	y6	17.1
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	1027.52	1	y9	18.2
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	914.43	1	y8	18.2
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	785.39	1	y7	18.2
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	1000.47	1	y8	21.4
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	872.41	1	y7	21.4
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	801.38	1	y6	21.4
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	1028.54	1	y9	16.3
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	713.39	1	y6	16.3
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	612.35	1	y5	16.3
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	770.42	1	y7	21.6
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	541.35	1	y5	21.6
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	428.26	1	y4	21.6
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	846.45	2	1208.62	1	y11	30.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	846.45	2	1107.57	1	y10	30.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	846.45	2	978.53	1	y9	30.3
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	763.40	1	y7	17.6
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	666.35	1	y6	17.6
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	579.31	1	y5	17.6
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	823.33	1	y7	17.2
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	736.29	1	y6	17.2
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	576.26	1	y5	17.2
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	1108.52	1	y9	19.2
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	894.42	1	y7	19.2
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	747.35	1	y6	19.2
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	1002.53	1	y8	20.6
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	631.37	1	y5	20.6

IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	504.24	1	y4	15.9
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	826.41	2	1066.54	1	y10	29.5
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	826.41	2	995.50	1	y9	29.5
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	826.41	2	924.47	1	y8	29.5
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	870.44	1	y8	20.2
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	769.39	1	y7	20.2
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	698.35	1	y6	20.2
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	571.80	2	769.36	1	y7	20.1
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	571.80	2	670.29	1	y6	20.1
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	571.80	2	599.25	1	y5	20.1
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	914.49	1	y10	21.0
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	843.46	1	y9	21.0
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	746.40	1	y8	21.0
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	746.38	1	y7	16.5
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	588.31	1	y5	16.5
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	475.23	1	y4	16.5
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	930.49	1	y9	25.6
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	859.45	1	y8	25.6
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	731.39	1	y7	25.6
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1154.58	1	y10	28.7
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1083.55	1	y9	28.7
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	913.44	1	y7	28.7
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	931.42	1	y8	18.6
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	834.36	1	y7	18.6
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	519.26	1	y4	18.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	719.39	1	y7	14.8
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	606.31	1	y6	14.8
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	434.22	1	y4	14.8
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	639.31	1	b6	14.8
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1187.58	1	y10	24.4
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1100.55	1	y9	24.4
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	971.50	1	y8	24.4
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	645.36	1	y5	14.6
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	516.31	1	y4	14.6
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	387.27	1	y3	14.6
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	864.43	2	1031.50	1	y10	30.9
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	864.43	2	960.46	1	y9	30.9
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	864.43	2	889.43	1	y8	30.9
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	899.49	1	y9	19.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	614.36	1	y6	19.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	543.32	1	y5	19.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	942.48	2	1208.66	1	y10	33.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	942.48	2	994.53	1	y8	33.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	942.48	2	831.47	1	y7	33.8
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1258.63	1	y11	27.3
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1058.52	1	y9	27.3
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	945.43	1	y8	27.3
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1277.64	1	y11	31.0
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1176.59	1	y10	31.0
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1077.52	1	y9	31.0
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	980.56	1	y8	23.7
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	780.44	1	y6	23.7
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	681.37	1	y5	23.7
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	818.48	1	y7	20.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	705.39	1	y6	20.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	542.33	1	y5	20.0
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	1048.50	1	y8	24.8
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	885.44	1	y7	24.8
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	738.37	1	y6	24.8
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	701.33	1	y5	15.6
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	586.30	1	y4	15.6
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	439.23	1	y3	15.6
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	703.39	1	y6	14.1
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	632.35	1	y5	14.1
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	446.27	1	y4	14.1
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	939.42	1	y8	20.2
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	727.34	1	y6	20.2
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	628.28	1	y5	20.2
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	674.37	1	y6	14.1
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	545.33	1	y5	14.1
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	416.29	1	y4	14.1
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	802.42	1	y6	16.8
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	689.34	1	y5	16.8
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	590.27	1	y4	16.8
FGNDVQHFK	sp|P62993|GRB2_HUMAN	546.27	2	773.39	1	y6	19.1
FGNDVQHFK	sp|P62993|GRB2_HUMAN	546.27	2	658.37	1	y5	19.1
FGNDVQHFK	sp|P62993|GRB2_HUMAN	546.27	2	431.24	1	y3	19.1
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	685.40	1	y6	16.8
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	586.33	1	y5	16.8
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	485.28	1	y4	16.8
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	924.43	1	y8	19.2
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	853.39	1	y7	19.2
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	738.37	1	y6	19.2
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1107.57	1	y11	25.8
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1020.54	1	y10	25.8
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	949.50	1	y9	25.8

ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	679.85	2	y11	20.6
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	642.43	1	y6	15.6
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	543.36	1	y5	15.6
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	430.28	1	y4	15.6
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	930.48	1	y8	20.4
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	817.39	1	y7	20.4
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	702.37	1	y6	20.4
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	889.46	1	y8	17.5
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	776.38	1	y7	17.5
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	662.34	1	y6	17.5
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	1005.56	1	y10	22.7
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	906.49	1	y9	22.7
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	819.46	1	y8	22.7
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	602.35	1	y6	15.4
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	503.28	1	y5	15.4
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	446.26	1	y4	15.4
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	1047.57	1	y10	24.7
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	948.50	1	y9	24.7
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	820.44	1	y8	24.7
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	788.46	1	y8	18.8
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	717.43	1	y7	18.8
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	618.36	1	y6	18.8
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	789.37	1	y7	16.1
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	676.29	1	y6	16.1
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	361.18	1	y3	16.1
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	974.52	1	y8	20.9
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	861.43	1	y7	20.9
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	748.35	1	y6	20.9
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	1338.61	1	y11	27.4
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	995.47	1	y8	27.4
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	866.43	1	y7	27.4
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	1060.55	1	y9	23.0
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	687.44	1	y6	23.0
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	574.36	1	y5	23.0
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	868.44	1	y7	18.0
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	755.36	1	y6	18.0
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	498.26	1	y4	18.0
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	823.43	1	y7	17.6
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	736.40	1	y6	17.6
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	665.36	1	y5	17.6
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	941.98	2	1293.65	1	y12	33.8
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	941.98	2	1196.59	1	y11	33.8
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	941.98	2	889.48	1	y8	33.8
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	746.42	1	y8	18.0
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	675.38	1	y7	18.0
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	574.33	1	y6	18.0
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	1096.53	1	y9	25.3
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	949.46	1	y8	25.3
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	723.37	1	y6	25.3
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	775.40	1	y7	17.6
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	612.34	1	y6	17.6
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	341.22	1	y3	17.6
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	933.48	1	y8	19.1
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	836.43	1	y7	19.1
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	650.35	1	y6	19.1
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	642.42	1	y6	11.9
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	399.76	2	y7	11.9
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	626.30	1	b5	11.9
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	515.32	1	y5	14.5
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	345.21	1	y3	14.5
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	701.30	1	b6	14.5
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	959.44	1	y8	26.5
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	858.39	1	y7	26.5
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	711.32	1	y6	26.5
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	944.48	1	y9	15.5
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	857.45	1	y8	15.5
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	720.39	1	y7	15.5
LFTGHPETLEK	sp|P68082|MYG_HORSE	636.34	2	1011.51	1	y9	22.5
LFTGHPETLEK	sp|P68082|MYG_HORSE	636.34	2	910.46	1	y8	22.5
LFTGHPETLEK	sp|P68082|MYG_HORSE	636.34	2	716.38	1	y6	22.5
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	817.36	1	y7	14.6
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	730.33	1	y6	14.6
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	629.28	1	y5	14.6
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	589.35	1	y5	12.4
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	532.32	1	y4	12.4
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	385.26	1	y3	12.4
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	943.51	1	y9	22.5
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	775.42	1	y7	22.5
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	646.38	1	y6	22.5
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	964.42	1	y8	18.9
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	865.35	1	y7	18.9
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	565.23	1	y4	18.9
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	738.38	2	974.51	1	y10	26.3
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	738.38	2	903.47	1	y9	26.3
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	738.38	2	790.38	1	y8	26.3
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	916.48	1	y8	20.0
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	716.36	1	y6	20.0
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	569.29	1	y5	20.0
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	696.39	1	y6	19.8
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	609.36	1	y5	19.8
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	446.30	1	y4	19.8

Supplemental Table S2D: MRM-MS transition list for each vendor platform, Agilent

S2D: Agilent instrument transition lists (displayed for light transitions)

Peptide Sequence	Protein Name	Precursor Mz	Precursor Charge	Product Mz	Product Charge	Fragment Ion	Collision Energy
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	888.47	1	y8	16.8
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	817.43	1	y7	16.8
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	659.36	1	y5	16.8
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	857.48	1	y9	14.3
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	758.42	1	y8	14.3
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	644.37	1	y7	14.3
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	674.38	1	y6	11.7
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	587.35	1	y5	11.7
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	472.32	1	y4	11.7
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	551.65	3	886.47	1	y8	15.1
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	551.65	3	757.43	1	y7	15.1
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	551.65	3	643.39	1	y6	15.1
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	823.39	1	y7	14.1
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	710.31	1	y6	14.1
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	563.24	1	y5	14.1
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	561.26	1	y5	11.6
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	432.22	1	y4	11.6
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	477.23	1	b4	11.6
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1178.65	1	y10	23.1
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	965.54	1	y8	23.1
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	818.47	1	y7	23.1
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	916.57	1	y8	20.2
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	815.52	1	y7	20.2
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	702.44	1	y6	20.2
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	817.94	2	y14	16.5
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	736.40	2	y13	16.5
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	679.86	2	y12	16.5
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	1064.50	1	y9	17.9
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	993.46	1	y8	17.9
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	865.41	1	y7	17.9
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	746.42	1	y8	12.5
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	647.35	1	y7	12.5
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	560.32	1	y6	12.5
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	708.40	1	y6	15.5
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	621.37	1	y5	15.5
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	522.30	1	y4	15.5
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	1135.57	1	y10	20.1
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	964.51	1	y8	20.1
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	801.45	1	y7	20.1
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	1004.50	1	y9	16.4
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	846.43	1	y7	16.4
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	616.38	1	y5	16.4
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	554.61	3	688.40	1	y6	15.2
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	554.61	3	559.36	1	y5	15.2
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	554.61	3	861.34	1	b9	15.2
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	780.36	1	y7	11.3
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	617.30	1	y6	11.3
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	489.24	1	y5	11.3
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	828.52	1	y7	14.0
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	715.43	1	y6	14.0
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	600.41	1	y5	14.0
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	805.41	1	y7	14.9
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	690.38	1	y6	14.9
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	575.35	1	y5	14.9
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	939.48	1	y9	17.1
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	852.45	1	y8	17.1
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	632.36	1	y6	17.1
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	772.43	1	y7	15.5
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	673.36	1	y6	15.5
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	806.33	1	b8	15.5
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	751.33	1	y6	13.1
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	636.30	1	y5	13.1
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	522.26	1	y4	13.1
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	805.38	1	y7	12.8
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	677.32	1	y6	12.8
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	576.27	1	y5	12.8
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	536.60	3	955.45	1	y8	14.5
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	536.60	3	721.35	1	y6	14.5
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	536.60	3	592.31	1	y5	14.5
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	1006.46	1	y9	20.6
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	893.37	1	y8	20.6
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	778.35	1	y7	20.6
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	820.49	1	y7	17.2
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	721.42	1	y6	17.2
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	622.36	1	y5	17.2
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	365.88	3	505.28	1	y4	8.4
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	365.88	3	406.21	1	y3	8.4
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	365.88	3	463.30	1	b4	8.4
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	794.42	1	y7	12.5
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	681.33	1	y6	12.5
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	594.30	1	y5	12.5
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	642.34	1	y5	14.1
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	529.26	1	y4	14.1
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	832.41	1	b7	14.1
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	877.46	1	y8	18.3
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	763.42	1	y7	18.3
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	664.35	1	y6	18.3
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	833.45	1	y7	13.9
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	732.40	1	y6	13.9
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	619.32	1	y5	13.9
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	531.77	2	y8	9.3
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	467.74	2	y7	9.3
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	418.20	2	y6	9.3
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	626.32	1	y4	11.5
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	556.76	2	y8	11.5
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	492.24	2	y7	11.5
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	754.37	1	y6	11.4
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	591.31	1	y5	11.4
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	903.47	1	y8	16.2
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	660.38	1	y6	16.2
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	589.35	1	y5	16.2
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	742.41	1	y8	12.2
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	629.33	1	y7	12.2
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	572.30	1	y6	12.2
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	842.93	2	y15	16.6
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	799.41	2	y14	16.6
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	742.87	2	y13	16.6
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	745.45	1	y7	11.7
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	646.38	1	y6	11.7
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	547.31	1	y5	11.7
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	834.43	1	y8	14.6
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	747.40	1	y7	14.6
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	660.37	1	y6	14.6
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	589.29	1	y5	10.2
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	460.25	1	y4	10.2
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	295.15	2	y5	10.2
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	823.48	1	y6	13.7
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	710.40	1	y5	13.7
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	597.31	1	y4	13.7
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	901.49	1	y7	15.3
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	754.42	1	y6	15.3
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	641.33	1	y5	15.3
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	938.42	1	y8	14.1
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	851.39	1	y7	14.1
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	637.26	1	y5	14.1
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	717.45	1	y7	11.5
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	646.41	1	y6	11.5
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	547.34	1	y5	11.5
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	847.41	1	y7	14.6
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	776.37	1	y6	14.6
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	590.29	1	y5	14.6
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	642.82	2	y10	13.1
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	585.80	2	y9	13.1
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	528.77	2	y8	13.1
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	720.37	1	y6	15.0
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	607.29	1	y5	15.0
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	928.39	1	b9	15.0
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	790.48	1	y7	11.0
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	643.41	1	y6	11.0
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	544.35	1	y5	11.0
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	855.39	1	y7	12.7
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	758.34	1	y6	12.7
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	643.31	1	y5	12.7
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	919.56	1	y9	15.7
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	818.51	1	y8	15.7
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	648.41	1	y6	15.7
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1103.61	1	y10	19.0
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1002.56	1	y9	19.0
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	446.27	1	y4	19.0
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	788.43	1	y8	12.5
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	717.39	1	y7	12.5
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	517.27	1	y5	12.5
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	959.50	1	y9	17.6
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	872.47	1	y8	17.6
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	662.34	1	y6	17.6
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	784.47	1	y7	16.4
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	656.41	1	y6	16.4
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	559.36	1	y5	16.4
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	692.40	1	y6	10.7
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	545.33	1	y5	10.7
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	432.25	1	y4	10.7
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	823.39	1	y7	11.9
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	708.37	1	y6	11.9
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	579.32	1	y5	11.9
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	910.45	1	y8	13.6
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	823.42	1	y7	13.6
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	579.35	1	y5	13.6
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	806.44	1	y8	12.8
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	735.40	1	y7	12.8
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	636.34	1	y6	12.8
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	914.43	1	y8	14.7
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	673.84	2	y12	14.7
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	595.79	2	y10	14.7
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	1000.47	1	y8	17.0
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	872.41	1	y7	17.0
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	801.38	1	y6	17.0
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	676.38	2	y12	12.8
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	627.86	2	y11	12.8
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	571.31	2	y10	12.8
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	984.55	1	y9	17.3
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	770.42	1	y7	17.3
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	656.37	1	y6	17.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	865.44	1	y8	15.5
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	778.41	1	y7	15.5
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	574.32	1	y5	15.5
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	763.40	1	y7	13.3
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	666.35	1	y6	13.3
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	432.25	1	y4	13.3
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	823.33	1	y7	13.0
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	736.29	1	y6	13.0
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	576.26	1	y5	13.0
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	752.90	2	y13	15.7
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	703.36	2	y12	15.7
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	667.85	2	y11	15.7
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	793.41	2	y13	17.2
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	736.87	2	y12	17.2

SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	478.23	1	y4	11.4
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	730.41	1	y6	11.7
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	617.33	1	y5	11.7
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	504.24	1	y4	11.7
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	795.42	1	y7	15.0
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	682.34	1	y6	15.0
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	611.30	1	y5	15.0
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	870.44	1	y8	15.9
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	769.39	1	y7	15.9
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	698.35	1	y6	15.9
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	670.29	1	y6	8.9
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	441.73	2	y8	8.9
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	385.18	2	y7	8.9
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	914.49	1	y10	16.6
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	843.46	1	y9	16.6
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	746.40	1	y8	16.6
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	746.38	1	y7	12.3
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	588.31	1	y5	12.3
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	475.23	1	y4	12.3
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	479.91	3	731.39	1	y7	12.5
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	479.91	3	630.35	1	y6	12.5
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	479.91	3	531.28	1	y5	12.5
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	536.61	3	913.44	1	y7	14.5
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	536.61	3	727.38	1	y5	14.5
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	536.61	3	564.31	1	y4	14.5
KPAEDEWGK	sp|P02792|FRIL_HUMAN	353.84	3	634.28	1	y5	7.9
KPAEDEWGK	sp|P02792|FRIL_HUMAN	353.84	3	390.21	1	y3	7.9
KPAEDEWGK	sp|P02792|FRIL_HUMAN	353.84	3	541.26	1	b5	7.9
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	719.39	1	y7	10.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	434.22	1	y4	10.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	639.31	1	b6	10.6
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1100.55	1	y9	19.9
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	971.50	1	y8	19.9
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	486.26	2	y8	19.9
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	645.36	1	y5	10.4
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	516.31	1	y4	10.4
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	459.21	1	b4	10.4
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	960.46	1	y9	16.0
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	889.43	1	y8	16.0
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	761.37	1	y6	16.0
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	899.49	1	y9	15.5
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	713.43	1	y7	15.5
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	614.36	1	y6	15.5
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	994.53	1	y8	17.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	831.47	1	y7	17.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	703.41	1	y6	17.8
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1058.52	1	y9	22.8
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	945.43	1	y8	22.8
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	782.37	1	y7	22.8
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	578.62	3	948.48	1	y8	16.0
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	578.62	3	847.43	1	y7	16.0
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	578.62	3	661.35	1	y6	16.0
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	446.58	3	780.44	1	y6	11.3
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	446.58	3	681.37	1	y5	11.3
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	446.58	3	518.31	1	y4	11.3
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	978.51	1	y8	15.7
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	818.48	1	y7	15.7
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	705.39	1	y6	15.7
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	465.90	3	1048.50	1	y8	12.0
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	465.90	3	885.44	1	y7	12.0
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	465.90	3	625.28	1	y5	12.0
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	814.41	1	y6	11.4
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	701.33	1	y5	11.4
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	586.30	1	y4	11.4
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	703.39	1	y6	9.9
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	632.35	1	y5	9.9
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	446.27	1	y4	9.9
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	939.42	1	y8	16.7
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	753.40	1	b8	16.7
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	852.47	1	b9	16.7
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	545.33	1	y5	10.0
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	416.29	1	y4	10.0
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	519.24	1	b4	10.0
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	689.34	1	y5	13.3
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	590.27	1	y4	13.3
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	623.31	2	y10	13.3
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	559.30	1	y4	8.3
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	431.24	1	y3	8.3
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	472.73	2	y8	8.3
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	685.40	1	y6	12.5
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	586.33	1	y5	12.5
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	485.28	1	y4	12.5
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	924.43	1	y8	15.0
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	853.39	1	y7	15.0
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	738.37	1	y6	15.0
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1107.57	1	y11	21.4
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	949.50	1	y9	21.4
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	475.25	2	y9	21.4

ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	679.85	2	y11	17.2
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	770.49	1	y7	11.4
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	642.43	1	y6	11.4
EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	543.36	1	y5	11.4
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	930.48	1	y8	16.1
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	817.39	1	y7	16.1
IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	702.37	1	y6	16.1
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	889.46	1	y8	13.3
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	776.38	1	y7	13.3
ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	662.34	1	y6	13.3
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	1005.56	1	y10	19.3
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	906.49	1	y9	19.3
EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	819.46	1	y8	19.3
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	602.35	1	y6	11.2
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	503.28	1	y5	11.2
EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	446.26	1	y4	11.2
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	1047.57	1	y10	20.2
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	948.50	1	y9	20.2
ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	820.44	1	y8	20.2
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	788.46	1	y8	15.3
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	717.43	1	y7	15.3
TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	618.36	1	y6	15.3
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	789.37	1	y7	11.9
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	676.29	1	y6	11.9
HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	605.25	1	y5	11.9
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	974.52	1	y8	16.6
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	861.43	1	y7	16.6
LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	748.35	1	y6	16.6
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	513.58	3	866.43	1	y7	13.7
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	513.58	3	622.36	1	y5	13.7
SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	513.58	3	544.26	1	b5	13.7
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	1060.55	1	y9	18.6
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	574.36	1	y5	18.6
QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	475.29	1	y4	18.6
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	868.44	1	y7	13.8
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	755.36	1	y6	13.8
YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	534.26	1	b4	13.8
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	823.43	1	y7	13.4
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	665.36	1	y5	13.4
WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	552.28	1	y4	13.4
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	889.48	1	y8	17.8
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	792.43	1	y7	17.8
LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	629.36	1	y6	17.8
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	746.42	1	y8	13.7
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	675.38	1	y7	13.7
DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	574.33	1	y6	13.7
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	1096.53	1	y9	20.9
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	949.46	1	y8	20.9
GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	723.37	1	y6	20.9
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	775.40	1	y7	13.3
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	612.34	1	y6	13.3
DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	515.28	1	y5	13.3
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	933.48	1	y8	14.9
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	836.43	1	y7	14.9
GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	650.35	1	y6	14.9
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	399.76	2	y7	8.4
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	458.21	1	b3	8.4
DWRPAITIK	sp|P63279|UBC9_HUMAN	367.21	3	626.30	1	b5	8.4
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	515.32	1	y5	11.0
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	554.24	1	b5	11.0
DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	701.30	1	b6	11.0
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	959.44	1	y8	22.0
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	858.39	1	y7	22.0
AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	612.26	1	y5	22.0
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	643.82	2	y12	12.0
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	586.80	2	y11	12.0
INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	472.74	2	y9	12.0
LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	716.38	1	y6	10.5
LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	579.79	2	y10	10.5
LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	506.26	2	y9	10.5
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	817.36	1	y7	11.1
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	523.74	2	y10	11.1
YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	488.22	2	y9	11.1
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	589.35	1	y5	8.3
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	532.32	1	y4	8.3
HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	385.26	1	y3	8.3
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	943.51	1	y9	18.1
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	846.46	1	y8	18.1
LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	775.42	1	y7	18.1
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	964.42	1	y8	14.6
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	865.35	1	y7	14.6
IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	808.33	1	y6	14.6
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	790.38	1	y8	12.9
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	651.35	2	y13	12.9
SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	537.29	2	y11	12.9
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	916.48	1	y8	15.7
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	716.36	1	y6	15.7
GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	569.29	1	y5	15.7
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	797.44	1	y7	15.5
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	696.39	1	y6	15.5
ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	609.36	1	y5	15.5

Supplemental Table S2E: MRM-MS transition list for each vendor platform, AB Sciex (QTRAP 5500)

S2E: AB Sciex (QTRAP 5500) instrument transition lists (displayed for light transitions)

Peptide Sequence	Protein Name	Precursor Mz	Precursor Charge	Product Mz	Product Charge	Fragment Ion	Collision Energy
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	888.47	1	y8	30.5
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	817.43	1	y7	30.5
ALQASALNAWR	sp|P09972|ALDOC_HUMAN	600.83	2	659.36	1	y5	30.5
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	758.42	1	y8	27.9
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	644.37	1	y7	27.9
AEVNGLAAQGK	sp|P09972|ALDOC_HUMAN	529.29	2	474.27	1	y5	27.9
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	674.38	1	y6	25.4
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	472.32	1	y4	25.4
ELSDIALR	sp|P09972|ALDOC_HUMAN	458.76	2	359.24	1	y3	25.4
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	1112.64	1	y10	38.6
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	999.56	1	y9	38.6
TPSALAILENANVLAR	sp|P09972|ALDOC_HUMAN	826.97	2	886.47	1	y8	38.6
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	823.39	1	y7	27.8
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	710.31	1	y6	27.8
QVLFSADDR	sp|P09972|ALDOC_HUMAN	525.76	2	563.24	1	y5	27.8
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	724.33	1	y6	25.2
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	561.26	1	y5	25.2
ALYEAGER	sp|P04083|ANXA1_HUMAN	454.73	2	432.22	1	y4	25.2
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1178.65	1	y10	36.8
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	1064.61	1	y9	36.8
GTDVNVFNTILTTR	sp|P04083|ANXA1_HUMAN	775.91	2	965.54	1	y8	36.8
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	987.61	1	y9	33.9
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	916.57	1	y8	33.9
GVDEATIIDILTK	sp|P04083|ANXA1_HUMAN	694.39	2	702.44	1	y6	33.9
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	1101.62	1	y10	29.8
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	853.45	2	y15	29.8
AAYLQETGKPLDETLK	sp|P04083|ANXA1_HUMAN	592.98	3	736.40	2	y13	29.8
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	1064.50	1	y9	31.6
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	993.46	1	y8	31.6
TPAQFDADELR	sp|P04083|ANXA1_HUMAN	631.80	2	865.41	1	y7	31.6
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	746.42	1	y8	26.1
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	647.35	1	y7	26.1
VLVSLSAGGR	sp|P09525|ANXA4_HUMAN	479.79	2	447.23	1	y5	26.1
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	708.40	1	y6	28.3
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	621.37	1	y5	28.3
GLGTDEDAIISVLAYR	sp|P09525|ANXA4_HUMAN	564.96	3	522.30	1	y4	28.3
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	964.51	1	y8	33.7
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	801.45	1	y7	33.7
DEGNYLDDALVR	sp|P09525|ANXA4_HUMAN	690.33	2	688.36	1	y6	33.7
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	1004.50	1	y9	30.0
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	846.43	1	y7	30.0
GLGTDDNTLIR	sp|P09525|ANXA4_HUMAN	587.81	2	731.40	1	y6	30.0
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	1131.62	1	y10	38.8
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	801.48	1	y7	38.8
GAGTDEGCLIEILASR	sp|P09525|ANXA4_HUMAN	831.41	2	446.27	1	y4	38.8
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	617.30	1	y6	25.0
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	489.24	1	y5	25.0
LYQAGEGR	sp|P20073|ANXA7_HUMAN	447.23	2	418.20	1	y4	25.0
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	828.52	1	y7	27.7
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	715.43	1	y6	27.7
SEIDLVQIK	sp|P20073|ANXA7_HUMAN	522.80	2	600.41	1	y5	27.7
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	963.47	1	y9	28.5
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	805.41	1	y7	28.5
GAGTDDSTLVR	sp|P20073|ANXA7_HUMAN	546.27	2	690.38	1	y6	28.5
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	939.48	1	y9	30.8
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	632.36	1	y6	30.8
EFSGYVESGLK	sp|P20073|ANXA7_HUMAN	608.30	2	533.29	1	y5	30.8
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	772.43	1	y7	28.3
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	673.36	1	y6	28.3
GFGTDEQAIVDVVANR	sp|P20073|ANXA7_HUMAN	564.28	3	459.27	1	y4	28.3
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	751.33	1	y6	26.7
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	636.30	1	y5	26.7
QIDNPDYK	sp|Q53G71|Q53G71_HUMAN	496.74	2	522.26	1	y4	26.7
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	805.38	1	y7	26.4
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	677.32	1	y6	26.4
GLQTSQDAR	sp|Q53G71|Q53G71_HUMAN	488.25	2	576.27	1	y5	26.4
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	1113.52	1	y10	37.8
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	955.45	1	y8	37.8
FYALSASFEPFSNK	sp|Q53G71|Q53G71_HUMAN	804.39	2	592.31	1	y5	37.8
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	1153.53	1	y10	34.3
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	893.37	1	y8	34.3
EQFLDGDGWTSR	sp|Q53G71|Q53G71_HUMAN	705.82	2	778.35	1	y7	34.3
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	1034.62	1	y9	30.8
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	820.49	1	y7	30.8
GQTLVVQFTVK	sp|Q53G71|Q53G71_HUMAN	610.36	2	721.42	1	y6	30.8
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	845.49	1	y7	28.6
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	732.41	1	y6	28.6
LHIVQVVCK	sp|O00299|CLIC1_HUMAN	548.32	2	633.34	1	y5	28.6
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	681.33	1	y6	26.1
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	594.30	1	y5	26.1
YLSNAYAR	sp|O00299|CLIC1_HUMAN	479.24	2	409.22	1	y3	26.1
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	642.34	1	y5	26.1
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	529.26	1	y4	26.1
IEEFLEAVLCPPR	sp|O00299|CLIC1_HUMAN	524.94	3	369.22	1	y3	26.1
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	1024.53	1	y9	31.9
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	877.46	1	y8	31.9
GVTFNVTTVDTK	sp|O00299|CLIC1_HUMAN	641.34	2	664.35	1	y6	31.9
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	619.32	1	y5	27.6
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	310.16	2	y5	27.6
GFTIPEAFR	sp|O00299|CLIC1_HUMAN	519.27	2	306.14	1	b3	27.6
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	649.32	1	y5	18.9
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	512.26	1	y4	18.9
IQVWHAEHR	sp|P15311|EZRI_HUMAN	392.54	3	467.74	2	y7	18.9
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	884.40	1	y6	22.2
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	755.36	1	y5	22.2
EDEVEEWQHR	sp|P15311|EZRI_HUMAN	452.87	3	626.32	1	y4	22.2
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	591.31	1	y5	25.0
SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	478.23	1	y4	25.0
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	903.47	1	y8	29.8
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	660.38	1	y6	29.8
LFDQAFGLPR	sp|P04792|HSPB1_HUMAN	582.31	2	589.35	1	y5	29.8
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	742.41	1	y8	25.8
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	629.33	1	y7	25.8
AQLGGPEAAK	sp|P04792|HSPB1_HUMAN	471.26	2	572.30	1	y6	25.8
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	872.47	1	y8	30.0
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	801.44	1	y7	30.0
VSLDVNHFAPDELTVK	sp|P04792|HSPB1_HUMAN	595.31	3	842.93	2	y15	30.0
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	646.38	1	y6	25.4
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	547.31	1	y5	25.4
DGVVEITGK	sp|P04792|HSPB1_HUMAN	459.25	2	418.27	1	y4	25.4
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	834.43	1	y8	28.2
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	660.37	1	y6	28.2
QLSSGVSEIR	sp|P04792|HSPB1_HUMAN	538.29	2	504.28	1	y4	28.2
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	704.32	1	y6	23.9
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	589.29	1	y5	23.9
EDELGDR	sp|Q14116|IL18_HUMAN	417.19	2	460.25	1	y4	23.9
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	710.40	1	y5	27.3
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	597.31	1	y4	27.3
SDIIFFQR	sp|Q14116|IL18_HUMAN	513.27	2	450.25	1	y3	27.3
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	901.49	1	y7	29.0
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	754.42	1	y6	29.0
TIFIISMYK	sp|Q14116|IL18_HUMAN	558.31	2	641.33	1	y5	29.0
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	938.42	1	y8	27.8
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	851.39	1	y7	27.8
ISTLSCENK	sp|Q14116|IL18_HUMAN	526.26	2	637.26	1	y5	27.8
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	717.45	1	y7	25.2
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	646.41	1	y6	25.2
GMAVTISVK	sp|Q14116|IL18_HUMAN	453.26	2	547.34	1	y5	25.2
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	847.41	1	y7	28.3
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	776.37	1	y6	28.3
DGGAWGTEQR	sp|P09382|LEG1_HUMAN	538.74	2	590.29	1	y5	28.3
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	670.34	1	y5	24.6
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	533.28	1	y4	24.6
DSNNLCLHFNPR	sp|P09382|LEG1_HUMAN	496.23	3	642.82	2	y10	24.6
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	720.37	1	y6	27.5
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	607.29	1	y5	27.5
FNAHGDANTIVCNSK	sp|P09382|LEG1_HUMAN	549.92	3	508.22	1	y4	27.5
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	643.41	1	y6	24.7
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	544.35	1	y5	24.7
SFVLNLGK	sp|P09382|LEG1_HUMAN	439.26	2	431.26	1	y4	24.7
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	855.39	1	y7	26.3
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	758.34	1	y6	26.3
LPDGYEFK	sp|P09382|LEG1_HUMAN	484.74	2	428.20	2	y7	26.3
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	818.51	1	y8	29.3
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	761.49	1	y7	29.3
CGTGIVGVFVK	sp|O00151|PDLI1_HUMAN	568.81	2	648.41	1	y6	29.3
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1103.61	1	y10	32.6
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	1002.56	1	y9	32.6
GCTDNLTLTVAR	sp|O00151|PDLI1_HUMAN	660.83	2	887.53	1	y8	32.6
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	788.43	1	y8	26.1
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	717.39	1	y7	26.1
VAASIGNAQK	sp|O00151|PDLI1_HUMAN	479.77	2	517.27	1	y5	26.1
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	959.50	1	y9	31.3
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	872.47	1	y8	31.3
VWSPLVTEEGK	sp|O00151|PDLI1_HUMAN	622.83	2	662.34	1	y6	31.3
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	913.51	1	y8	30.0
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	784.47	1	y7	30.0
DFEQPLAISR	sp|O00151|PDLI1_HUMAN	588.31	2	656.41	1	y6	30.0
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	692.40	1	y6	24.4
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	545.33	1	y5	24.4
GLFIIDGK	sp|P32119|PRDX2_HUMAN	431.76	2	432.25	1	y4	24.4
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	708.37	1	y6	25.5
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	579.32	1	y5	25.5
TDEGIAYR	sp|P32119|PRDX2_HUMAN	462.72	2	409.22	1	y3	25.5
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	910.45	1	y8	27.3
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	823.42	1	y7	27.3
LSEDYGVLK	sp|P32119|PRDX2_HUMAN	512.27	2	694.38	1	y6	27.3
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	806.44	1	y8	26.5
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	735.40	1	y7	26.5
ATAVVDGAFK	sp|P32119|PRDX2_HUMAN	489.77	2	636.34	1	y6	26.5
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	1027.52	1	y9	27.1
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	914.43	1	y8	27.1
DYGVYLEDSGHTLR	sp|Q13162|PRDX4_HUMAN	542.26	3	785.39	1	y7	27.1
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	1000.47	1	y8	30.7
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	872.41	1	y7	30.7
LVQAFQYTDK	sp|Q13162|PRDX4_HUMAN	606.82	2	801.38	1	y6	30.7
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	1028.54	1	y9	24.2
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	676.38	2	y12	24.2
IPLLSDLTHQISK	sp|Q13162|PRDX4_HUMAN	488.95	3	627.86	2	y11	24.2
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	770.42	1	y7	30.9
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	656.37	1	y6	30.9
QITLNDLPVGR	sp|Q13162|PRDX4_HUMAN	613.35	2	541.35	1	y5	30.9
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	865.44	1	y8	28.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	668.84	2	y12	28.3
ELLQTELSGFLDAQK	sp|P23297|S10A1_HUMAN	564.63	3	554.29	2	y10	28.3
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	763.40	1	y7	27.0
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	666.35	1	y6	27.0
ELPSFVGEK	sp|P29034|S10A2_HUMAN	503.27	2	579.31	1	y5	27.0
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	736.29	1	y6	26.6
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	576.26	1	y5	26.6
YSCQEGDK	sp|P29034|S10A2_HUMAN	493.70	2	448.20	1	y4	26.6
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	1108.52	1	y9	28.6
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	894.42	1	y7	28.6
AMVALIDVFHQYSGR	sp|P04271|S100B_HUMAN	569.63	3	747.35	1	y6	28.6
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	1002.53	1	y8	30.8
ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	622.83	2	y10	30.8

SGYLSSER	sp|P15311|EZRI_HUMAN	449.72	2	391.19	1	y3	25.0	ELINNELSHFLEEIK	sp|P04271|S100B_HUMAN	609.99	3	558.31	2	y9	30.8
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	801.45	1	y7	25.3	EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	642.43	1	y6	25.1
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	730.41	1	y6	25.3	EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	543.36	1	y5	25.1
IALLEEAR	sp|P15311|EZRI_HUMAN	457.77	2	617.33	1	y5	25.3	EQVIAALR	sp|P54727|RD23B_HUMAN	450.27	2	430.28	1	y4	25.1
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	795.42	1	y7	27.6	IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	930.48	1	y8	29.7
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	682.34	1	y6	27.6	IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	817.39	1	y7	29.7
SQEQLAAELAEYTAK	sp|P15311|EZRI_HUMAN	551.28	3	611.30	1	y5	27.6	IDIDPEETVK	sp|P54727|RD23B_HUMAN	579.80	2	702.37	1	y6	29.7
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	870.44	1	y8	29.5	ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	889.46	1	y8	26.9
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	769.39	1	y7	29.5	ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	776.38	1	y7	26.9
YLTAEAFGFK	sp|Q16658|FSCN1_HUMAN	573.80	2	698.35	1	y6	29.5	ILNDDTALK	sp|P54727|RD23B_HUMAN	501.78	2	662.34	1	y6	26.9
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	670.29	1	y6	18.3	EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	1005.56	1	y10	34.0
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	441.73	2	y8	18.3	EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	906.49	1	y9	34.0
FLIVAHDDGR	sp|Q16658|FSCN1_HUMAN	381.54	3	385.18	2	y7	18.3	EQANAVSEAVVSSVNTV	sp|O76070|SYUG_HUMAN	668.68	3	819.46	1	y8	34.0
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	914.49	1	y10	30.3	EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	602.35	1	y6	24.8
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	843.46	1	y9	30.3	EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	503.28	1	y5	24.8
YLAPSGPSGTLK	sp|Q16658|FSCN1_HUMAN	595.82	2	746.40	1	y8	30.3	EGVVGAVEK	sp|O76070|SYUG_HUMAN	444.25	2	446.26	1	y4	24.8
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	746.38	1	y7	25.9	ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	1047.57	1	y10	33.9
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	588.31	1	y5	25.9	ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	948.50	1	y9	33.9
VTGTLDANR	sp|Q16658|FSCN1_HUMAN	473.75	2	475.23	1	y4	25.9	ENVVQSVTSVAEK	sp|O76070|SYUG_HUMAN	695.36	2	820.44	1	y8	33.9
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	930.49	1	y9	34.8	TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	717.43	1	y7	28.0
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	731.39	1	y7	34.8	TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	618.36	1	y6	28.0
LSCFAQTVSPAEK	sp|Q16658|FSCN1_HUMAN	719.36	2	531.28	1	y5	34.8	TVEEAENIAVTSGVVR	sp|O76070|SYUG_HUMAN	558.63	3	517.31	1	y5	28.0
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1154.58	1	y10	37.8	HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	676.29	1	y6	25.6
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	1083.55	1	y9	37.8	HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	476.21	1	y4	25.6
LGGPEAGLGEYLFER	sp|P02792|FRIL_HUMAN	804.41	2	913.44	1	y7	37.8	HIAEDADR	sp|P09493|TPM1_HUMAN	463.72	2	361.18	1	y3	25.6
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	931.42	1	y8	27.9	LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	974.52	1	y8	30.2
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	834.36	1	y7	27.9	LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	861.43	1	y7	30.2
KPAEDEWGK	sp|P02792|FRIL_HUMAN	530.26	2	519.26	1	y4	27.9	LVIIESDLER	sp|P09493|TPM1_HUMAN	593.84	2	748.35	1	y6	30.2
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	606.31	1	y6	24.3	SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	995.47	1	y8	36.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	639.31	1	b6	24.3	SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	866.43	1	y7	36.6
HLTASEAK	sp|P39748|FEN1_HUMAN	428.73	2	710.35	1	b7	24.3	SIDDLEDELYAQK	sp|P09493|TPM1_HUMAN	769.86	2	751.40	1	y6	36.6
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1187.58	1	y10	33.6	QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	1060.55	1	y9	32.3
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	1100.55	1	y9	33.6	QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	687.44	1	y6	32.3
WSEPNEEELIK	sp|P39748|FEN1_HUMAN	687.33	2	971.50	1	y8	33.6	QLEDELVSLQK	sp|P09493|TPM1_HUMAN	651.35	2	574.36	1	y5	32.3
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	645.36	1	y5	24.1	YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	868.44	1	y7	27.4
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	516.31	1	y4	24.1	YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	755.36	1	y6	27.4
SIEEIVR	sp|P39748|FEN1_HUMAN	423.24	2	387.27	1	y3	24.1	YLQETYSK	sp|O00762|UBE2C_HUMAN	516.26	2	627.30	1	y5	27.4
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	1031.50	1	y10	29.0	WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	823.43	1	y7	27.0
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	889.43	1	y8	29.0	WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	736.40	1	y6	27.0
QLQQAQAAGAEQEVEK	sp|P39748|FEN1_HUMAN	576.62	3	632.32	1	y5	29.0	WSALYDVR	sp|O00762|UBE2C_HUMAN	505.26	2	552.28	1	y4	27.0
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	899.49	1	y9	29.1	LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	889.48	1	y8	31.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	614.36	1	y6	29.1	LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	792.43	1	y7	31.8
LIADVAPSAIR	sp|P39748|FEN1_HUMAN	563.33	2	543.32	1	y5	29.1	LSLEFPSGYPYNAPTVK	sp|O00762|UBE2C_HUMAN	628.32	3	629.36	1	y6	31.8
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	994.53	1	y8	31.8	DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	746.42	1	y8	27.4
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	831.47	1	y7	31.8	DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	675.38	1	y7	27.4
FQDGDLTLYQSNTILR	sp|P09211|GSTP1_HUMAN	628.65	3	703.41	1	y6	31.8	DPAATSVAAAR	sp|O00762|UBE2C_HUMAN	515.27	2	574.33	1	y6	27.4
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1171.60	1	y10	36.5	GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	1096.53	1	y9	34.5
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	1058.52	1	y9	36.5	GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	949.46	1	y8	34.5
YISLIYTNYEAGK	sp|P09211|GSTP1_HUMAN	767.89	2	945.43	1	y8	36.5	GISAFPESDNLFK	sp|O00762|UBE2C_HUMAN	712.86	2	852.41	1	y7	34.5
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	1077.52	1	y9	40.1	DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	515.32	1	y5	21.5
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	948.48	1	y8	40.1	DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	345.21	1	y3	21.5
EEVVTVETWQEGSLK	sp|P09211|GSTP1_HUMAN	867.43	2	847.43	1	y7	40.1	DHPFGFVAVPTK	sp|P63279|UBC9_HUMAN	438.90	3	701.30	1	b6	21.5
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	879.51	1	y7	33.0	GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	836.43	1	y7	28.5
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	780.44	1	y6	33.0	GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	650.35	1	y6	28.5
PPYTVVYFPVR	sp|P09211|GSTP1_HUMAN	669.37	2	681.37	1	y5	33.0	GTPWEGGLFK	sp|P63279|UBC9_HUMAN	546.28	2	467.25	2	y8	28.5
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	818.48	1	y7	29.3	DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	775.40	1	y7	27.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	705.39	1	y6	29.3	DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	612.34	1	y6	27.0
ASCLYGQLPK	sp|P09211|GSTP1_HUMAN	568.79	2	542.33	1	y5	29.3	DDYPSSPPK	sp|P63279|UBC9_HUMAN	503.23	2	341.22	1	y3	27.0
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	1048.50	1	y8	34.0	DWRPAITIK	sp|P63279|UBC9_HUMAN	550.32	2	492.80	2	y8	28.7
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	885.44	1	y7	34.0	DWRPAITIK	sp|P63279|UBC9_HUMAN	550.32	2	840.44	1	b7	28.7
FSLYFLAYEDK	sp|Q04760|LGUL_HUMAN	698.35	2	738.37	1	y6	34.0	DWRPAITIK	sp|P63279|UBC9_HUMAN	550.32	2	953.52	1	b8	28.7
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	701.33	1	y5	25.1	AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	1087.50	1	y9	35.7
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	586.30	1	y4	25.1	AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	959.44	1	y8	35.7
SLDFYTR	sp|Q04760|LGUL_HUMAN	451.22	2	439.23	1	y3	25.1	AGLCQTFVYGGCR	sp|P00974|BPT1_BOVIN	744.84	2	858.39	1	y7	35.7
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	703.39	1	y6	23.6	INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	720.39	1	y7	23.0
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	632.35	1	y5	23.6	INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	643.82	2	y12	23.0
IAWALSR	sp|Q04760|LGUL_HUMAN	408.74	2	446.27	1	y4	23.6	INDISHTQSVSAK	sp|P41160|LEP_MOUSE	467.24	3	586.80	2	y11	23.0
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	628.28	1	y5	30.1	LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	716.38	1	y6	20.7
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	470.22	2	y8	30.1	LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	579.79	2	y10	20.7
GFGHIGIAVPDVYSACK	sp|Q04760|LGUL_HUMAN	597.63	3	753.40	1	b8	30.1	LFTGHPETLEK	sp|P68082|MYG_HORSE	424.56	3	506.26	2	y9	20.7
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	674.37	1	y6	23.7	YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	817.36	1	y7	21.6
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	545.33	1	y5	23.7	YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	523.74	2	y10	21.6
FEELGVK	sp|Q04760|LGUL_HUMAN	411.22	2	416.29	1	y4	23.7	YLASASTMDHAR	sp|P02687|MBP_BOVIN	441.54	3	488.22	2	y9	21.6
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	802.42	1	y6	24.9	HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	589.35	1	y5	22.0
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	689.34	1	y5	24.9	HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	532.32	1	y4	22.0
FNSLNELVDYHR	sp|P62993|GRB2_HUMAN	502.92	3	590.27	1	y4	24.9	HGFLPR	sp|P02687|MBP_BOVIN	363.71	2	385.26	1	y3	22.0
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	773.39	1	y6	17.4	LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	943.51	1	y9	31.8
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	658.37	1	y5	17.4	LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	846.46	1	y8	31.8
FGNDVQHFK	sp|P62993|GRB2_HUMAN	364.51	3	559.30	1	y4	17.4	LSEPAELTDAVK	sp|P07288|KLK3_HUMAN	636.84	2	775.42	1	y7	31.8
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	685.40	1	y6	26.2	IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	964.42	1	y8	28.3
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	586.33	1	y5	26.2	IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	865.35	1	y7	28.3
NYVTPVNR	sp|P62993|GRB2_HUMAN	481.76	2	485.28	1	y4	26.2	IVGGWECEK	sp|P07288|KLK3_HUMAN	539.26	2	808.33	1	y6	28.3
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	924.43	1	y8	28.6	SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	974.51	1	y10	24.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	853.39	1	y7	28.6	SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	790.38	1	y8	24.4
ATADDELSFK	sp|P62993|GRB2_HUMAN	548.76	2	738.37	1	y6	28.6	SSDLVALSGGHTFGK	sp|P00433|PER1A_ARMRU	492.59	3	703.35	1	y7	24.4
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1107.57	1	y11	35.0	GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	916.48	1	y8	29.3
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	1020.54	1	y10	35.0	GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	829.45	1	y7	29.3
ESESAPGDFSLSVK	sp|P62993|GRB2_HUMAN	726.85	2	949.50	1	y9	35.0	GYSIFSYATK	sp|P02741|CRP_HUMAN	568.78	2	716.36	1	y6	29.3
								ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	912.47	1	y8	29.2
								ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	797.44	1	y7	29.2
								ESDTSYVSLK	sp|P02741|CRP_HUMAN	564.77	2	696.39	1	y6	29.2

Supplemental Table S3: Blinded Sample Concentrations for Phases II and III

Phase II (Study 9.1), concentrations in fmol

	Run #	Run #	Run #	Run #	Run #	Run #
	01blind	02blind	03blind	04blind	05blind	06blind
1	1.75	1.75	72	19.4	0.105	0.105
	07blind	08blind	09blind	10blind	11blind	12blind
2	19.4	19.4	1.75	0.105	72	72
	13blind	14blind	15blind	16blind	17blind	18blind
3	72	72	0.105	1.75	19.4	19.4
	19blind	20blind	21blind	22blind	23blind	24blind
4	0.105	0.105	19.4	72	1.75	1.75

Phase III (Study 9.2), concentrations in fmol

	Run #	Run #	Run #	Run #	Run #	Run #
1	01a_blind	02a_blind	03a_blind	01b_blind	02b_blind	03b_blind
	4	25	75	4	25	75
2	04a_blind	05a_blind	06a_blind	04b_blind	05b_blind	06b_blind
	75	4	25	75	4	25
3	07a-T1_blind	08a-T1_blind	09a-T1_blind	07b-T1_blind	08b-T1_blind	09b-T1_blind
	25	75	4	25	75	4
4	07a-T2_blind	08a-T2_blind	09a-T2_blind	07b-T2_blind	08b-T2_blind	09b-T2_blind
	25	75	4	25	75	4

Supplemental Table S6: LODs and LOQs of Study 7 peptides, comparing Study 7 and Phase II

LOD and LOQ are reported in fmol transition ID: 'precursor charge state.product ion type.product ion charge state'

study	site	peptide	transition.id	LOD	LOQ
study-9.1	19	AGLCQT	2.y9.1	0.18	0.55
study-9.1	19	HGFLPR	2.y5.1	0.07	0.22
study-9.1	19	INDISH	3.y11.2	0.05	0.15
study-9.1	19	IVGGWE	2.y8.1	0.21	0.63
study-9.1	19	LFTGHP	3.y6.1	0.03	0.08
study-9.1	19	LSEPAE	2.y9.1	0.20	0.61
study-9.1	19	SSDLVA	3.y8.1	0.09	0.26
study-9.1	19	YLASAS	3.y9.2	0.07	0.21
study-9.1	32	AGLCQT	2.y8.1	0.20	0.61
study-9.1	32	HGFLPR	2.y4.1	0.05	0.15
study-9.1	32	INDISH	3.y12.2	0.10	0.29
study-9.1	32	IVGGWE	2.y7.1	0.19	0.58
study-9.1	32	LFTGHP	3.y9.2	0.10	0.29
study-9.1	32	LSEPAE	2.y8.1	0.14	0.43
study-9.1	32	SSDLVA	3.y10.1	0.17	0.52
study-9.1	32	YLASAS	3.y10.2	0.14	0.43
study-9.1	52A	AGLCQT	2.y9.1	0.80	2.41
study-9.1	52A	HGFLPR	2.y4.1	0.37	1.11
study-9.1	52A	INDISH	3.y7.1	0.42	1.27
study-9.1	52A	IVGGWE	2.y8.1	0.55	1.66
study-9.1	52A	LFTGHP	3.y10.2	0.41	1.23
study-9.1	52A	LSEPAE	2.y8.1	0.53	1.58
study-9.1	52A	SSDLVA	3.y10.1	0.41	1.24
study-9.1	52A	YLASAS	3.y9.2	0.31	0.93
study-9.1	54	AGLCQT	2.y9.1	0.16	0.48
study-9.1	54	HGFLPR	2.y5.1	0.08	0.23
study-9.1	54	INDISH	3.y12.2	0.07	0.20
study-9.1	54	IVGGWE	2.y7.1	0.16	0.47
study-9.1	54	LFTGHP	3.y9.2	0.07	0.22
study-9.1	54	LSEPAE	2.y9.1	0.08	0.25
study-9.1	54	SSDLVA	3.y10.1	0.07	0.20
study-9.1	54	YLASAS	3.y10.2	0.06	0.17
study-9.1	56A	AGLCQT	2.y8.1	0.23	0.68
study-9.1	56A	HGFLPR	2.y5.1	0.07	0.21
study-9.1	56A	INDISH	3.y7.1	0.05	0.15
study-9.1	56A	IVGGWE	2.y8.1	0.12	0.36
study-9.1	56A	LFTGHP	3.y6.1	0.06	0.19
study-9.1	56A	LSEPAE	2.y9.1	0.16	0.47
study-9.1	56A	SSDLVA	3.y8.1	0.08	0.24
study-9.1	56A	YLASAS	3.y7.1	0.06	0.17
study-9.1	56B60	AGLCQT	2.y5.1	0.38	1.13
study-9.1	56B60	HGFLPR	2.y5.1	0.07	0.20
study-9.1	56B60	INDISH	3.y12.2	0.08	0.23
study-9.1	56B60	IVGGWE	2.y7.1	0.18	0.53
study-9.1	56B60	LFTGHP	3.y10.2	0.08	0.25
study-9.1	56B60	LSEPAE	2.y9.1	0.15	0.46
study-9.1	56C	AGLCQT	2.y6. 1	0.33	0.99
study-9.1	56C	HGFLPR	2.y4. 1	0.03	0.09
study-9.1	56C	INDISH	3.y8. 1	0.07	0.20
study-9.1	56C	IVGGWE	2.y8. 1	0.13	0.40
study-9.1	56C	LFTGHP	2.y6. 1	0.12	0.36
study-9.1	56C	LSEPAE	2.y9. 1	0.08	0.23
study-9.1	56C	SSDLVA	2.y10. 1	0.17	0.50
study-9.1	56C	YLASAS	3.y7. 1	0.07	0.21
study-9.1	65A	AGLCQT	2.y8.1	0.15	0.46
study-9.1	65A	HGFLPR	2.y4.1	0.04	0.12
study-9.1	65A	INDISH	3.y12.2	0.08	0.23
study-9.1	65A	IVGGWE	2.y6.1	0.31	0.94
study-9.1	65A	LFTGHP	3.y6.1	0.09	0.26
study-9.1	65A	LSEPAE	3.y11.2	0.29	0.87
study-9.1	65A	SSDLVA	3.y10.1	0.07	0.20
study-9.1	65A	YLASAS	3.y7.1	0.05	0.16
study-9.1	73A	AGLCQT	2.y7.1	0.06	0.17
study-9.1	73A	HGFLPR	2.y5.1	0.07	0.22
study-9.1	73A	INDISH	3.y9.1	0.93	2.80
study-9.1	73A	IVGGWE	2.y7.1	0.11	0.34
study-9.1	73A	LFTGHP	2.y6.1	0.47	1.42
study-9.1	73A	LSEPAE	2.y9.1	0.04	0.12
study-9.1	73A	SSDLVA	2.y10.1	0.12	0.36
study-9.1	73A	YLASAS	3.y7.1	0.46	1.38
study-9.1	73	AGLCQT	2.y9.1	0.28	0.85
study-9.1	73	HGFLPR	2.y5.1	0.11	0.33
study-9.1	73	INDISH	3.y12.2	0.07	0.21
study-9.1	73	IVGGWE	2.y7.1	0.20	0.61
study-9.1	73	LFTGHP	3.y9.2	0.07	0.21
study-9.1	73	LSEPAE	2.y9.1	0.14	0.43
study-9.1	73	SSDLVA	3.y10.1	0.12	0.36
study-9.1	73	YLASAS	3.y10.2	0.08	0.23
study-9.1	86A	AGLCQT	2.y7. 1	0.12	0.37
study-9.1	86A	HGFLPR	2.y5. 1	0.03	0.10
study-9.1	86A	INDISH	3.y7. 1	0.04	0.11
study-9.1	86A	IVGGWE	2.y8. 1	0.12	0.35
study-9.1	86A	LFTGHP	2.y9. 1	0.04	0.13
study-9.1	86A	LSEPAE	2.y9. 1	0.06	0.19
study-9.1	86A	SSDLVA	2.y10. 1	0.23	0.68
study-9.1	86A	YLASAS	3.y7. 1	0.02	0.05
study-9.1	86	AGLCQT	2.y8. 1	0.19	0.56
study-9.1	86	HGFLPR	2.y5. 1	0.09	0.27
study-9.1	86	INDISH	3.y12. 2	0.10	0.29
study-9.1	86	IVGGWE	2.y8. 1	0.22	0.67
study-9.1	86	LFTGHP	3.y9. 2	0.08	0.25
study-9.1	86	LSEPAE	2.y9. 1	0.09	0.26

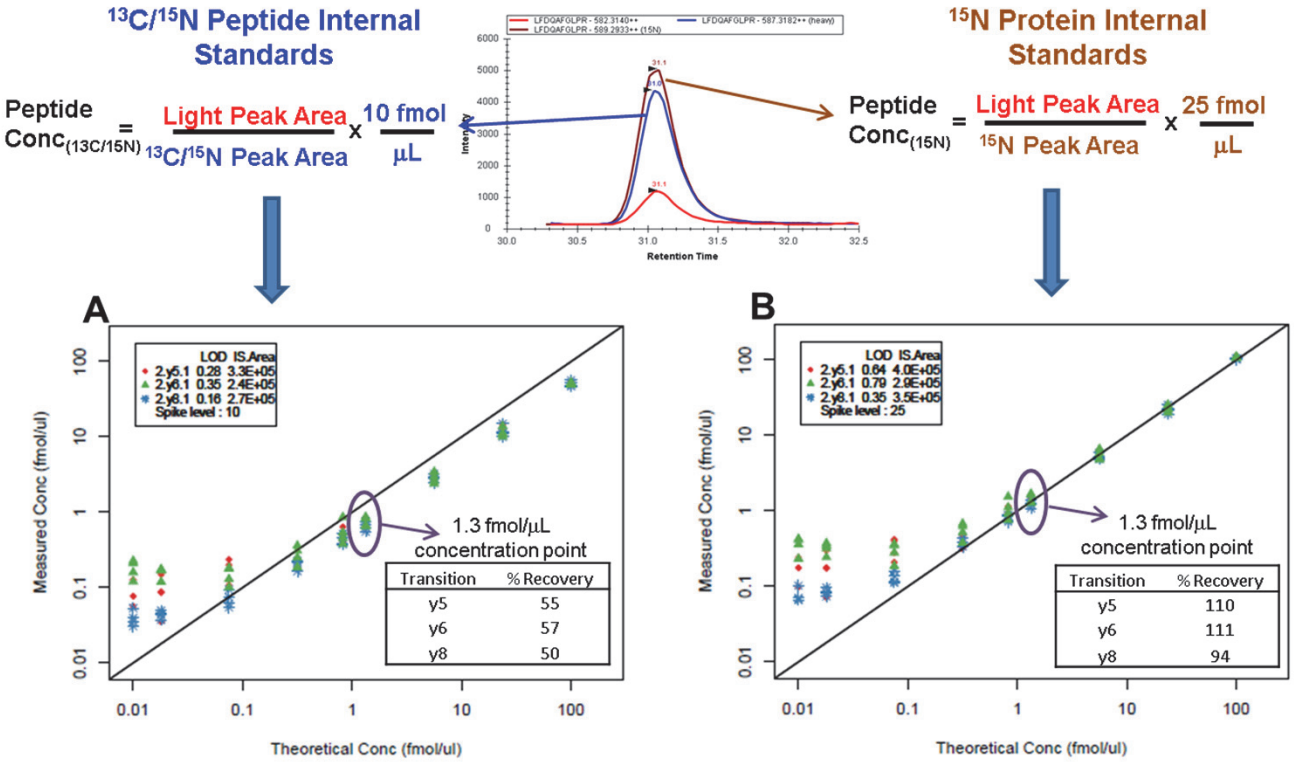
study-9.1	56B60	SSDLVA	3.y8.1	0.10	0.29
study-9.1	56B60	YLASAS	3.y7.1	0.03	0.10
study-9.1	56B90	AGLCQT	2.y7. 1	0.26	0.77
study-9.1	56B90	HGFLPR	2.y4. 1	0.06	0.19
study-9.1	56B90	INDISH	3.y12. 2	0.07	0.21
study-9.1	56B90	IVGGWE	2.y8. 1	0.14	0.41
study-9.1	56B90	LFTGHP	3.y9. 2	0.07	0.20
study-9.1	56B90	LSEPAE	2.y9. 1	0.07	0.22
study-9.1	56B90	SSDLVA	3.y8. 1	0.09	0.26
study-9.1	56B90	YLASAS	3.y9. 2	0.04	0.12
study-9.1	86	SSDLVA	3.y8. 1	0.07	0.20
study-9.1	86	YLASAS	3.y10. 2	0.08	0.24
study-9.1	90	AGLCQT	2.y5.1	0.34	1.02
study-9.1	90	HGFLPR	2.y5.1	0.07	0.21
study-9.1	90	INDISH	3.y9.2	0.16	0.49
study-9.1	90	IVGGWE	2.y7.1	0.22	0.66
study-9.1	90	LFTGHP	3.y10.2	0.10	0.30
study-9.1	90	LSEPAE	2.y9.1	0.10	0.29
study-9.1	90	SSDLVA	3.y8.1	0.45	1.36
study-9.1	90	YLASAS	3.y10.2	598.71	1796.14

Supplemental Table S6 - continued

study	site	peptide	transition.id	LOD	LOQ
study-7.1	56	LSEPAE	37tr3_A	0.78	2.34
study-7.1	56	IVGGWE	161tr2_A	0.93	2.78
study-7.1	56	SSDLVA	166tr2_A	0.46	1.39
study-7.1	56	INDISH	167tr2_A	0.20	0.61
study-7.1	56	HGFLPR	169tr3_A	0.34	1.03
study-7.1	56	YLASAS	170tr2_A	0.25	0.74
study-7.1	56	LFTGHP	171tr1_A	0.39	1.18
study-7.1	56	AGLCQT	173tr2_A	0.81	2.42
study-7.1	52	LSEPAE	37tr3_A	0.24	0.70
study-7.1	52	IVGGWE	161tr2_A	0.41	1.22
study-7.1	52	SSDLVA	166tr3_A	0.21	0.62
study-7.1	52	INDISH	NA	NA	NA
study-7.1	52	HGFLPR	169tr3_A	0.61	1.84
study-7.1	52	YLASAS	170tr2_A	0.25	0.74
study-7.1	52	LFTGHP	171tr1_A	0.48	1.45
study-7.1	52	AGLCQT	173tr2_A	0.61	1.83
study-7.1	73	LSEPAE	37tr3_A	0.47	1.40
study-7.1	73	IVGGWE	161tr2_A	0.69	2.08
study-7.1	73	SSDLVA	166tr2_A	0.83	2.49
study-7.1	73	INDISH	167tr2_A	0.21	0.63
study-7.1	73	HGFLPR	169tr3_A	0.29	0.87
study-7.1	73	YLASAS	170tr2_A	0.16	0.49
study-7.1	73	LFTGHP	171tr1_A	0.27	0.82
study-7.1	73	AGLCQT	173tr2_A	0.84	2.51
study-7.1	95	LSEPAE	37tr3_A	0.26	0.78
study-7.1	95	IVGGWE	161tr2_A	0.56	1.67
study-7.1	95	SSDLVA	166tr2_A	0.27	0.82
study-7.1	95	INDISH	167tr3_A	0.14	0.42
study-7.1	95	HGFLPR	169tr3_A	0.12	0.35
study-7.1	95	YLASAS	170tr2_A	0.14	0.41
study-7.1	95	LFTGHP	171tr1_A	0.31	0.92
study-7.1	95	AGLCQT	173tr2_A	0.30	0.90
study-7.1	86	LSEPAE	37tr3_A	0.13	0.38
study-7.1	86	IVGGWE	161tr2_A	0.48	1.45
study-7.1	86	SSDLVA	166tr3_A	0.13	0.39
study-7.1	86	INDISH	167tr1_A	1.29	3.86
study-7.1	86	HGFLPR	169tr3_A	0.29	0.88
study-7.1	86	YLASAS	170tr2_A	0.37	1.11
study-7.1	86	LFTGHP	171tr3_A	0.15	0.44
study-7.1	86	AGLCQT	173tr3_A	0.44	1.31
study-7.1	54	LSEPAE	37tr3_A	0.59	1.78
study-7.1	54	IVGGWE	161tr1_A	0.60	1.79
study-7.1	54	SSDLVA	166tr3_A	0.15	0.44
study-7.1	54	INDISH	167tr2_A	0.06	0.19
study-7.1	54	HGFLPR	169tr3_A	0.18	0.53
study-7.1	54	YLASAS	170tr3_A	0.25	0.75
study-7.1	54	LFTGHP	171tr2_A	0.26	0.79
study-7.1	54	AGLCQT	173tr2_A	0.42	1.27
study-7.1	19	LSEPAE	37tr3_A	0.40	1.21
study-7.1	19	IVGGWE	161tr2_A	0.93	2.79
study-7.1	19	SSDLVA	166tr2_A	0.28	0.84
study-7.1	19	INDISH	167tr2_A	0.26	0.79
study-7.1	19	HGFLPR	169tr3_A	0.28	0.84
study-7.1	19	YLASAS	170tr3_A	0.30	0.91
study-7.1	19	LFTGHP	171tr1_A	0.23	0.69
study-7.1	19	AGLCQT	173tr2_A	0.50	1.51
study-7.1	65	LSEPAE	37tr2_A	0.35	1.05
study-7.1	65	IVGGWE	161tr1_A	1.03	3.08
study-7.1	65	SSDLVA	166tr3_A	0.29	0.87
study-7.1	65	INDISH	167tr3_A	0.76	2.27
study-7.1	65	HGFLPR	169tr2_A	0.56	1.67
study-7.1	65	YLASAS	170tr3_A	0.29	0.86
study-7.1	65	LFTGHP	171tr3_A	0.11	0.31
study-7.1	65	AGLCQT	173tr3_A	0.69	2.08

study 7.1 transition ID: 'internal transition numbering' & 'transition 1 (tr1) / transition 2 (tr2) / transition 3 (tr2)'

Supplemental Figure S1. Proteins as Internal Standards Improve Accuracy over 13C/15N peptides



Supplemental Figure S1. Protein Internal Standards Improve Quantitative Accuracy. In the same set of data files, either the 13C/15N peptide or the U15N protein peak areas are used to calculate the measured concentration of the target peptides. The effect of using the U15N protein peak areas is improved quantitative accuracy in that the true recovered peptide amount (and hence, protein amount) is determined and no assumptions need to be made regarding protein digestion or sample handling.

Supplemental Table S7: Median Peptide Recoveries Phase III

(with 13C/15N and U15N standards)

based on 13C15N internal peptide standards

peptide	52A	54	56A	56B90	56C	65A	86	86A
AAYLQETGKPLDETLK	0.0468	0.0361	0.0349	0.0266	NA	0.0092	0.0513	0.0395
AEVNGLAAQGK	0.3364	0.2839	0.1978	0.1510	0.2819	0.0322	0.2937	0.4162
ALQASALNAWR	0.5624	0.5216	0.6479	0.4769	0.4156	0.1485	0.5053	0.6522
ALYEAGER	0.3637	0.4555	0.2995	0.3302	0.0000	0.1330	0.4643	0.5816
AMVALIDVFHQYSGR	0.6020	0.6813	0.5767	0.6110	0.5260	0.1436	0.7198	0.6690
AQLGGPEAAK	0.4462	NA	0.1887	0.2160	0.3532	0.0848	0.4419	0.3367
ASCLYGQLPK	0.5720	0.5897	0.4843	0.5009	0.5393	0.1534	0.7139	0.6300
ATADDELSFK	0.9515	0.9049	0.7284	0.6759	0.6778	0.2353	0.9115	0.8733
ATAVVDGAFK	0.6644	0.5646	0.4060	0.4239	0.5356	0.1373	0.6717	0.5791
DDYPSSPPK	0.0903	0.3016	0.0711	0.0785	NA	0.0233	0.1090	0.2416
DEGNYLDDALVR	0.1591	0.3124	0.1140	0.1006	0.0711	0.0276	0.1298	0.3873
DFEQPLAISR	0.6888	0.6681	0.4124	0.4465	0.5574	0.1017	0.5750	0.7941
DGGAWGTEQR	0.6748	0.3938	0.4293	0.4102	0.6288	0.2380	0.6500	0.6279
DGVVEITGK	0.1740	0.1982	0.0857	0.0895	0.0665	0.0211	0.1232	0.2229
DHPFGFVAVPTK	0.1513	0.2239	0.1373	0.1341	0.1231	0.0212	0.2112	0.2708
DPAATSVAAAR	0.1359	0.1376	0.0690	0.0660	0.0007	0.0224	0.1195	0.2555
DSNNLCLHFNPR	0.8383	0.7223	0.5126	0.4895	0.7812	0.1427	0.7059	0.6437
DYGVYLEDSGHTLR	0.1480	0.2538	0.1112	0.1414	0.1313	0.1261	0.1638	0.2684
EDEVEEWQHR	0.0256	0.0367	0.0166	0.0349	0.0108	0.0074	0.0267	0.0313
EEVVTVETWQEGSLK	0.1376	0.1603	0.0786	0.1889	0.0000	0.0049	0.0734	0.1836
EFSGYVESGLK	0.3858	0.2835	0.2616	0.2974	0.2477	0.1193	0.3954	0.3387
EGVVGAVEK	0.3520	0.2505	0.1660	0.1867	0.2621	0.0783	0.3349	0.3261
ELPSFVGEK	0.3124	0.3374	0.1915	0.1853	0.2008	0.0347	0.2752	0.5214
ELSDIALR	0.2997	0.4906	0.1490	0.3165	0.3875	0.1346	0.4936	0.4880
ENVVQSVTSVAEK	0.0592	0.0885	0.0396	0.0479	0.0290	0.0064	0.0534	0.0722
EQFLDGDGWTSR	0.8087	0.5596	0.4705	2.5312	0.8945	0.2000	0.5881	0.6945
EQVIAALR	0.7309	0.6424	0.5181	0.5353	0.5523	0.1834	0.7192	0.6877
ESESAPGDFSLSVK	0.9961	0.8972	0.5814	0.7169	0.7886	0.2572	0.8948	0.5450
FEELGVK	0.3214	0.2241	0.1767	0.1699	0.1227	0.0640	0.2151	0.2581
FGNDVQHFK	0.8072	0.6574	0.5394	0.5519	0.4065	0.1781	0.5715	0.5423
FLIVAHDDGR	0.0588	0.0533	0.0318	0.3421	0.0252	0.0063	0.0183	0.0611
FNAHGDANTIVCNSK	0.7961	0.6580	0.5225	0.4590	0.6385	0.1681	0.7293	0.7045
FNSLNELVDYHR	0.7934	0.8432	0.6941	0.7050	0.7736	0.1899	0.8706	0.8949
FQDGDLTLYQSNTILR	1.3024	1.3325	0.7158	0.7802	2.2048	0.3288	2.5322	1.2234
FSLYFLAYEDK	0.0239	0.0715	0.0303	11.6013	0.0530	0.0093	0.0321	0.0717
FYALSASFEPFSNK	0.4709	0.4132	0.3821	0.4804	0.5879	0.1432	0.4418	0.5317
GAGTDDSTLVR	0.3544	0.2355	0.2056	0.2025	0.2081	0.0878	0.2470	0.3379
GAGTDEGCLIEILASR	0.7103	0.6123	0.5246	0.5284	0.8319	0.1355	0.6790	0.5850
GCTDNLTLTVAR	0.5527	0.5207	0.3220	0.3165	0.4659	0.1107	0.3355	0.6428
GFGHIGIAVPDVYSACK	0.8116	0.7252	0.4706	0.5463	0.7961	0.1282	0.7399	0.7549
GFTIPEAFR	0.4507	0.5535	0.3423	0.4270	0.3732	0.1188	0.5090	0.7757
GISAFPESDNLFK	0.5193	0.4881	0.2638	0.3167	0.3621	0.0942	0.4369	0.4954
GLFIIDGK	0.4843	0.4489	0.3731	0.3423	NA	0.0966	0.4961	0.5221
GMAVTISVK	0.4097	0.4176	0.2396	0.3078	0.3283	0.1208	0.4241	0.4488
GTDVNVFNTILTTR	0.5973	0.4958	0.3031	0.2763	0.3899	0.1088	0.4235	0.6647
GTPWEGGLFK	6.3928	7.4179	5.6646	5.7449	10.2460	1.6436	6.9877	8.4849

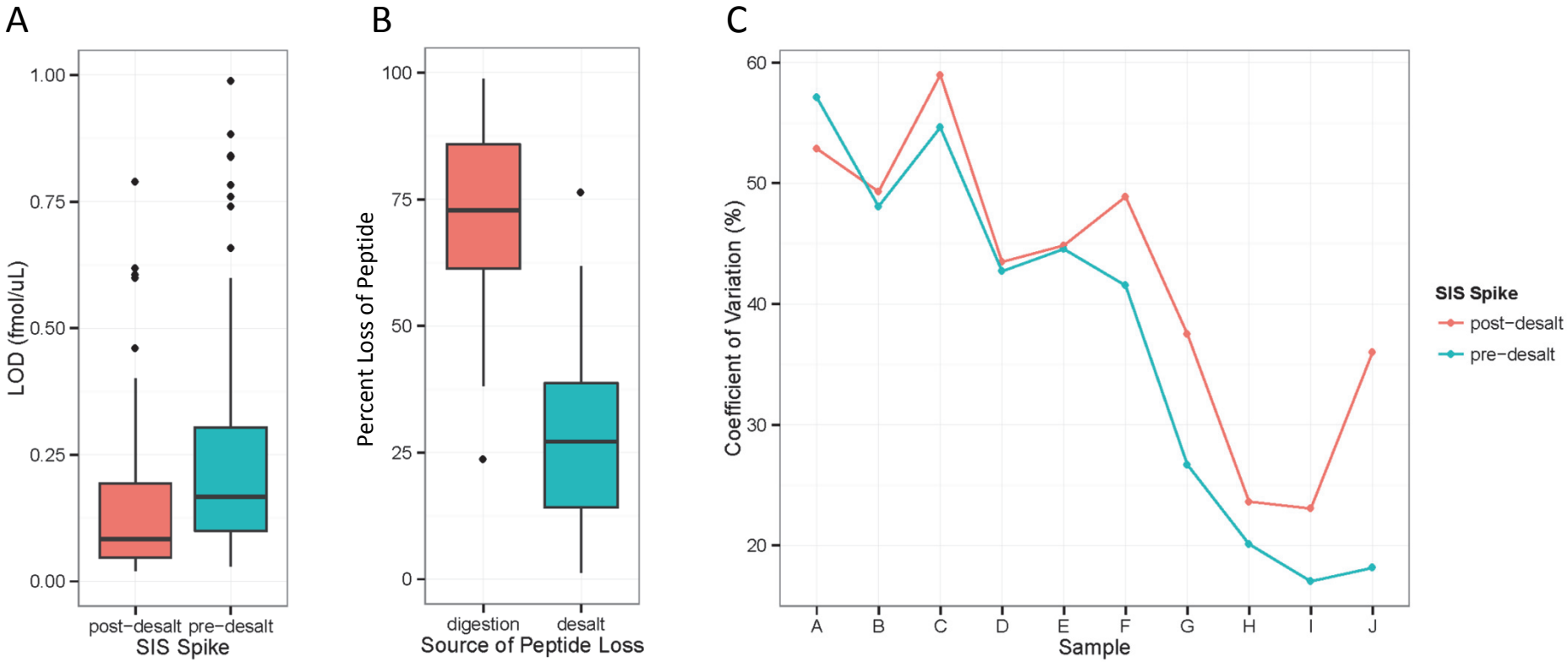
GVTFNVTTVDTK	0.2827	0.3394	0.2078	0.2243	0.1294	0.0766	0.2936	0.3576
IALLEEAR	0.1797	0.2639	0.1241	0.2017	0.1664	0.0849	0.1809	0.3027
IAWALSR	0.1499	0.0597	0.1350	0.2179	0.1683	0.1085	0.2081	0.0967
IDIDPEETVK	1.0151	NA	0.6676	0.6730	0.8848	0.1749	0.9432	0.8784
IEEFLEAVLCPPR	0.0433	0.1214	0.0365	0.0295	0.0187	-0.0076	0.0262	0.1558
ILNDDTALK	0.4207	0.4476	0.2618	0.2815	0.1952	0.0646	0.4031	0.6490
IPLLSDLTHQISK	0.0528	0.1118	0.0656	0.0501	0.0348	0.0328	0.0873	0.0581
IQVWHAEHR	0.3548	0.4818	0.3122	NA	0.0169	0.1308	0.5057	0.4518
ISTLSCENK	0.5032	0.4819	0.2672	0.2434	0.3818	0.1161	0.5768	0.4205
LFDQAFGLPR	0.4847	0.4473	0.3536	0.3713	0.2851	0.1027	0.4986	0.4606
LHIVQVVCK	0.3810	0.4253	0.2320	0.2333	0.3357	0.0751	0.3534	0.4797
LIADVAPSAIR	0.3220	0.2953	0.1608	0.1549	0.2113	0.0448	0.2256	0.2745
LPDGYEFK	0.0332	0.0309	0.0369	0.0465	0.0000	0.0981	0.0393	0.0144
LSCFAQTVSPAEK	0.0342	0.0266	0.0197	0.0507	NA	0.0103	0.0160	0.0264
LSEDYGVLK	0.0436	0.0649	0.0283	0.0488	0.2807	0.0535	0.0695	0.3839
LSLEFPSGYPYNAPTVK	0.2852	0.4761	0.4564	0.4051	0.4896	0.1039	0.4985	0.5187
LVIIESDLER	0.0615	0.0903	0.0421	0.0496	0.0270	0.0060	0.0499	0.1142
LVQAFQYTDK	0.1496	0.2729	0.1377	0.1073	0.1437	0.0592	0.1659	0.2393
LYQAGEGR	0.3839	0.3350	0.1429	0.2244	0.2963	0.1242	0.3629	0.0078
NYVTPVNR	0.7729	0.6442	0.4336	0.4724	0.7349	0.1435	0.6637	0.6858
QIDNPDYK	0.6717	0.5641	0.3830	0.3802	0.0000	0.1755	0.7973	0.6841
QITLNDLPVGR	0.1468	0.2787	0.1561	0.1191	0.1777	0.0796	0.1744	0.2886
QLEDELVSLQK	0.2239	0.2892	0.1670	0.1655	0.1520	0.0377	0.1757	0.3734
QLQQAQAAGAEQEVEK	0.2628	0.1830	0.1271	0.1038	0.1543	0.0423	0.1546	0.1872
QLSSGVSEIR	0.4998	0.4509	0.3521	0.3316	0.3681	0.1231	0.5122	0.4971
QVLFSADDR	0.4425	0.5246	0.4380	0.3360	0.4566	0.1339	0.5623	0.5946
SDIIFFQR	0.6324	0.6475	0.5289	0.5325	0.5538	0.2093	0.6415	0.6015
SEIDLVQIK	0.3678	0.3422	0.2239	0.2241	0.2274	0.1028	0.2932	0.3763
SFVLNLGK	0.6121	0.5582	0.3345	0.4921	0.4305	0.1474	0.6521	0.6277
SGYLSSER	0.4647	0.4738	0.2596	0.2630	0.2811	0.1329	0.4513	0.4547
SIDDLEDELYAQK	0.1347	0.1134	0.0808	0.1190	0.0995	0.0294	0.1190	0.0874
SIEEIVR	0.2108	0.1807	0.1018	0.1042	0.1992	0.0487	0.1230	0.1833
SLDFYTR	0.5366	0.5185	0.3535	0.3722	0.3720	0.1473	0.4686	0.5501
SQEQLAAELAEYTAK	0.4488	0.4166	0.3812	0.3482	0.3977	0.0962	0.4447	0.3992
TDEGIAYR	0.5641	0.4791	0.3178	0.3513	0.4979	0.1430	0.5935	0.5409
TIFIISMYK	0.3552	0.3985	0.2943	0.3130	0.3239	0.0908	0.3542	0.4119
TPAQFDADELR	0.1998	0.1604	0.1923	0.1779	0.1313	0.1271	0.1794	0.0870
TPSALAILENANVLAR	0.8243	0.6926	0.5347	0.3824	0.7105	0.1311	0.7242	0.7465
TVEEAENIAVTSGVVR	0.2471	0.2447	0.1860	0.1672	0.1686	0.0647	0.3648	0.2671
VAASIGNAQK	0.4199	0.4483	0.1117	0.1197	0.3854	0.0729	0.3582	0.3003
VLVSLSAGGR	0.1329	0.2347	0.0920	0.0890	0.0733	0.0188	0.1417	0.3535
VSLDVNHFAPDELTVK	0.5239	0.4523	0.3422	0.3293	0.3176	0.0752	0.4230	0.4196
VTGTLDANR	0.0176	0.0492	0.0041	0.0035	NA	0.0055	0.0058	0.0178
VWSPLVTEEGK	0.0636	0.0674	0.0490	0.0460	0.0393	0.0122	0.0447	0.0640
WSALYDVR	0.4426	0.4900	0.2707	0.3055	0.3960	0.1634	0.4576	0.5122
WSEPNEEELIK	0.3971	0.2909	0.1638	0.1782	0.2285	0.1009	0.2436	0.2229
YLAPSGPSGTLK	0.0635	0.0400	0.0213	0.0135	0.0167	0.0083	0.0242	0.0382
YLQETYSK	0.5897	0.5428	0.2934	0.3208	0.3395	0.1485	0.5086	0.4909
YLSNAYAR	0.4686	0.5392	0.2998	0.3609	0.3923	0.1530	0.5335	0.5310
YLTAEAFGFK	0.0423	0.0289	0.0132	0.0179	0.0175	0.0076	0.0264	0.0346

based on U-15N digested protein standards

peptide	52A	54	56A	56B90	56C	65A	86	86A
AAYLQETGKPLDETLK	1.2012	0.6740	1.4427	0.9723	NA	0.7407	1.3852	0.2411
AEVNGLAAQGK	0.9052	0.8016	1.4954	0.6687	0.9348	1.1590	1.2960	1.3454
ALQASALNAWR	1.2379	1.2454	1.7887	1.2730	0.8050	1.2390	1.0166	1.2051
ALYEAGER	0.7762	0.8624	0.9860	0.9766	0.0000	0.9689	0.9928	0.9867
AMVALIDVFHQYSGR	1.0964	1.1287	1.0096	1.0091	0.5632	1.1579	1.1594	1.0514
AQLGGPEAAK	0.9346	1.0019	0.8879	0.9681	0.4390	1.0127	1.0557	0.7150
ASCLYGQLPK	1.0200	0.9229	0.8836	0.9220	0.5212	0.9771	1.0462	0.8866
ATADDELSFK	1.5046	1.7692	2.0140	1.5275	0.9056	1.5698	1.7121	1.5169
ATAVVDGAFK	0.9733	1.0130	0.9253	0.7786	0.5976	1.0359	0.9963	0.9159
DDYPSSPPK	1.2415	0.7588	1.0694	1.0518	NA	1.0763	1.1752	0.8533
DEGNYLDDALVR	0.9108	0.9778	1.0247	0.9727	0.5978	0.9707	0.9204	0.8152
DFEQPLAISR	1.6241	1.5424	1.4871	1.5455	1.5770	1.3062	1.6005	1.7126
DGGAWGTEQR	1.0167	0.8713	1.0632	0.9555	0.8266	1.0861	1.3094	0.9886
DGVVEITGK	0.9771	1.0141	0.9443	0.9554	0.6769	1.0315	1.0284	0.9279
DHPFGFVAVPTK	1.0603	0.9376	1.1389	1.0679	0.5951	0.9554	1.1596	1.0599
DPAATSVAAAR	1.1459	0.8001	1.0247	1.0071	0.0080	1.0386	1.1292	1.1182
DSNNLCLHFNPR	1.1140	0.9650	0.9395	1.1179	0.7714	1.1061	1.1371	0.9919
DYGVYLEDSGHTLR	1.2450	0.8332	0.9109	0.8711	1.0826	1.0075	0.9628	1.0783
EDEVEEWQHR	0.9886	1.3285	0.8053	1.0622	0.6068	4.1781	1.0333	1.0780
EEVVTVETWQEGSLK	2.1726	1.0444	1.1756	1.5744	0.0000	0.6094	0.9691	0.7040
EFSGYVESGLK	0.9341	0.7679	0.8366	0.8795	0.4814	0.8654	0.8965	0.7847
EGVVGAVEK	1.0225	0.9166	1.0146	1.0112	0.6073	1.0869	0.9993	1.0507
ELPSFVGEK	0.8881	0.9488	0.8794	0.8439	0.8300	0.9614	0.9092	0.9357
ELSDIALR	0.6860	1.3142	0.4905	1.1388	0.7544	1.0941	1.2445	1.1798
ENVVQSVTSVAEK	0.9013	0.9230	0.7227	0.9099	0.5692	1.2726	0.8274	0.7263
EQFLDGDGWTSR	1.1855	1.0672	1.0351	2.9189	1.2008	1.0864	1.0567	1.1812
EQVIAALR	1.7207	1.6991	1.8179	1.8822	1.2360	1.7998	1.9594	1.7557
ESESAPGDFSLSVK	1.5957	1.6558	1.4075	1.6549	1.0324	1.6861	1.3976	1.2621
FEELGVK	1.0594	1.0195	1.0974	1.1076	0.4933	1.0360	1.0619	1.1611
FGNDVQHFK	1.5825	1.8165	1.6058	1.4976	0.9226	1.2292	1.1340	1.3803
FLIVAHDDGR	0.0778	0.0528	0.0679	NA	0.0232	0.1237	0.0279	0.0851
FNAHGDANTIVCNSK	0.9628	1.0326	1.2309	0.9125	0.5360	1.0782	1.1522	0.9852
FNSLNELVDYHR	1.4038	1.5523	1.8303	1.6570	1.1017	1.6762	1.6469	1.6478
FQDGDLTLYQSNTILR	0.8473	0.9375	0.8977	0.7609	0.7725	0.9678	0.7924	0.8934
FSLYFLAYEDK	0.1183	0.7777	0.4512	1.3832	1.3415	0.9026	0.9147	0.5918
FYALSASFEPFSNK	1.1341	1.0952	1.1281	1.1567	1.0971	1.2252	1.0179	1.0962
GAGTDDSTLVR	0.9574	0.6545	1.0397	0.9943	0.5289	0.8838	0.8572	0.8716
GAGTDEGCLIEILASR	0.8796	0.9474	0.8741	0.9007	0.7661	0.9974	0.9298	0.8155
GCTDNLTLTVAR	0.4527	1.2791	0.3311	0.6925	1.3800	1.4035	1.2679	1.7011
GFGHIGIAVPDVYSACK	0.9673	0.9048	0.8964	0.8727	0.6896	0.8981	0.8897	0.8745
GFTIPEAFR	0.8557	0.9033	0.8099	1.0278	0.5346	0.9359	1.0623	1.2552
GISAFPESDNLFK	1.1013	1.0790	0.8241	1.0664	0.8477	1.0271	1.0037	1.0027
GLFIIDGK	1.0272	0.9506	0.9522	0.9508	NA	1.0200	1.0416	1.0087
GMAVTISVK	0.7074	0.7555	0.8076	0.7289	0.3641	0.6567	0.7708	0.6723
GTDVNVFNTILTTR	0.9978	0.9372	0.9392	0.9027	0.7210	1.0398	0.8994	0.9887
GTPWEGGLFK	1.0735	1.0473	1.0321	1.0107	0.6397	1.0037	1.2041	1.1162
GVTFNVTTVDTK	0.9880	1.0426	0.9667	0.9192	0.4541	1.1592	0.8900	0.9265
IALLEEAR	0.7470	1.0495	0.8084	1.0774	0.6095	0.6829	0.8159	1.0218

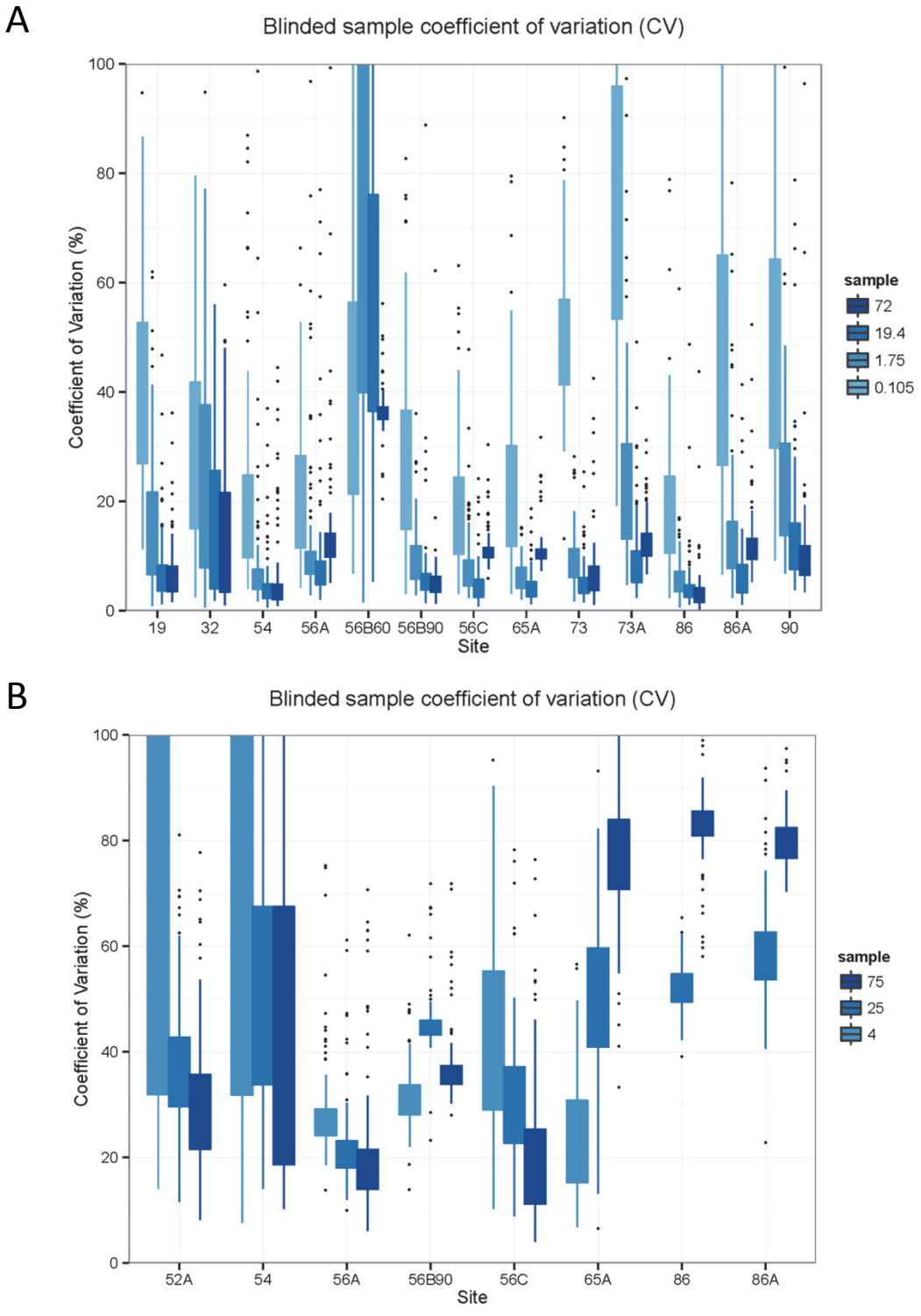
IAWALSR	0.3676	0.2903	0.8804	0.7010	0.5856	1.0383	1.0012	0.9787
IDIDPEETVK	1.9953	1.7545	2.0356	1.9347	1.5559	1.8239	2.0720	1.8273
IEEFLEAVLCPPR	0.8006	1.2472	1.1834	2.9208	1.3479	9.9512	0.3672	1.7036
ILNDDTALK	1.9674	1.5616	1.8706	1.8207	1.6770	1.7684	2.2781	1.7525
IPLLSDLTHQISK	1.2672	0.8579	1.0565	0.8937	1.1367	0.9782	1.2122	0.9485
IQVWHAEHR	0.9224	1.4156	1.2073	0.0928	0.0309	1.3422	1.0295	1.0750
ISTLSCENK	0.7812	0.6096	0.8322	0.7275	0.5376	0.7547	0.8537	0.6495
LFDQAFGLPR	0.9827	0.9016	0.8745	0.9516	0.4548	1.1020	0.9557	0.9363
LHIVQVVCK	1.1896	1.1150	1.0790	0.8830	0.7619	1.1304	0.9647	0.9108
LIADVAPSAIR	0.7046	0.6101	0.5192	0.5318	0.4145	0.6165	0.6369	0.5853
LPDGYEFK	0.2283	0.0640	0.4347	0.3319	0.0000	0.5792	0.3568	0.0617
LSCFAQTVSPAEK	0.0843	0.0576	0.0366	0.1954	0.0130	0.1078	0.0367	0.0469
LSEDYGVLK	0.7592	0.6978	0.7815	0.7847	0.7231	0.8883	0.9663	0.8623
LSLEFPSGYPYNAPTVK	0.8870	1.0600	1.0807	0.9529	0.8043	1.1000	0.9471	0.9444
LVIIESDLER	0.9086	0.9592	0.8946	0.8327	0.6495	0.6188	0.7840	0.7720
LVQAFQYTDK	1.5874	1.0236	1.1703	0.9748	0.8656	0.9146	1.0001	0.7369
LYQAGEGR	0.7545	0.8412	0.6954	0.8393	0.4761	0.8056	0.7604	0.1023
NYVTPVNR	1.7396	1.5828	1.5588	1.6257	1.2905	1.6054	1.7218	1.6389
QIDNPDYK	0.9651	1.0049	1.3751	1.2580	0.0000	1.0599	1.3313	1.1032
QITLNDLPVGR	1.2699	0.8797	1.4964	1.0741	0.7462	0.7789	0.1958	0.9493
QLEDELVSLQK	1.1313	0.9510	1.1236	1.0357	0.8625	0.9527	0.9350	0.8655
QLQQAQAAGAEQEVEK	0.7313	0.5274	0.7375	0.5517	0.4566	0.6569	0.6394	0.5838
QLSSGVSEIR	1.0240	1.0808	1.1635	0.9885	0.5052	0.9938	1.1690	0.9725
QVLFSADDR	1.1429	1.2664	1.8429	1.2357	1.0888	1.2282	1.3503	1.2464
SDIIFFQR	0.7564	0.8051	0.8281	0.8152	0.4516	0.8369	0.8188	0.7716
SEIDLVQIK	0.8326	0.9572	0.7762	0.7931	0.5750	0.8148	0.8551	0.8060
SFVLNLGK	0.9260	0.9234	0.7805	0.9686	0.3566	0.9625	1.0937	0.9590
SGYLSSER	1.0739	1.2165	1.0561	1.0817	0.5487	1.1122	1.0995	1.0311
SIDDLEDELYAQK	0.9332	0.9345	0.8071	1.0262	0.6450	0.8232	0.9352	0.6976
SIEEIVR	0.5981	0.6281	0.5451	0.5179	0.5440	0.5422	0.5997	0.6305
SLDFYTR	0.9631	1.1721	1.0001	1.0512	0.4469	1.1640	1.1139	1.0909
SQEQLAAELAEYTAK	1.0685	1.0926	1.1006	1.0019	0.8311	1.1156	1.0937	0.9617
TDEGIAYR	0.9266	0.9225	0.9868	1.0178	0.7910	1.0332	1.0704	1.0195
TIFIISMYK	0.7146	0.7711	0.8530	0.6935	0.3735	0.7321	0.7317	0.6909
TPAQFDADELR	1.0590	1.0163	1.1434	0.9591	0.6417	0.9622	1.0640	0.5947
TPSALAILENANVLAR	1.3628	1.3516	1.2664	1.1710	1.1762	1.2863	1.0746	1.3039
TVEEAENIAVTSGVVR	0.8585	0.9475	0.9162	0.7415	0.6855	0.9294	0.9347	1.1098
VAASIGNAQK	3.1889	2.8618	3.1378	3.3696	2.9087	3.6142	3.9388	0.7634
VLVSLSAGGR	0.8755	0.8981	0.8991	0.8071	0.7169	0.8320	1.0993	0.8880
VSLDVNHFAPDELTVK	1.0014	0.9988	0.8322	0.8379	0.5295	0.8534	0.8656	0.8399
VTGTLDANR	0.0750	0.1414	0.0570	0.0337	NA	0.0910	0.0436	0.0807
VWSPLVTEEGK	2.3777	2.3302	2.1852	1.9838	1.8891	2.2956	2.1154	2.2607
WSALYDVR	1.0226	1.0791	0.9785	0.9168	0.6877	1.0210	1.2726	0.7401
WSEPNEEELIK	0.6895	0.5527	0.5781	0.5901	0.3234	0.6968	0.5340	0.4052
YLAPSGPSGTLK	0.0807	0.0618	0.0555	0.0591	0.0177	0.0583	0.0417	0.0625
YLQETYSK	1.0290	0.7585	0.9600	0.8790	0.5534	0.9596	1.0697	0.8462
YLSNAYAR	0.8744	1.0271	1.0278	0.9021	0.4773	0.9391	1.0218	0.8930
YLTAEAFGFK	0.0834	0.0484	0.0355	0.0483	0.0248	0.0628	0.0457	0.0471

Supplemental Figure S2. LOD, Source of Peptide Loss, and CV plots for Pre- vs. Post-desalt SIS spikes for sites 56A, 56B and 65



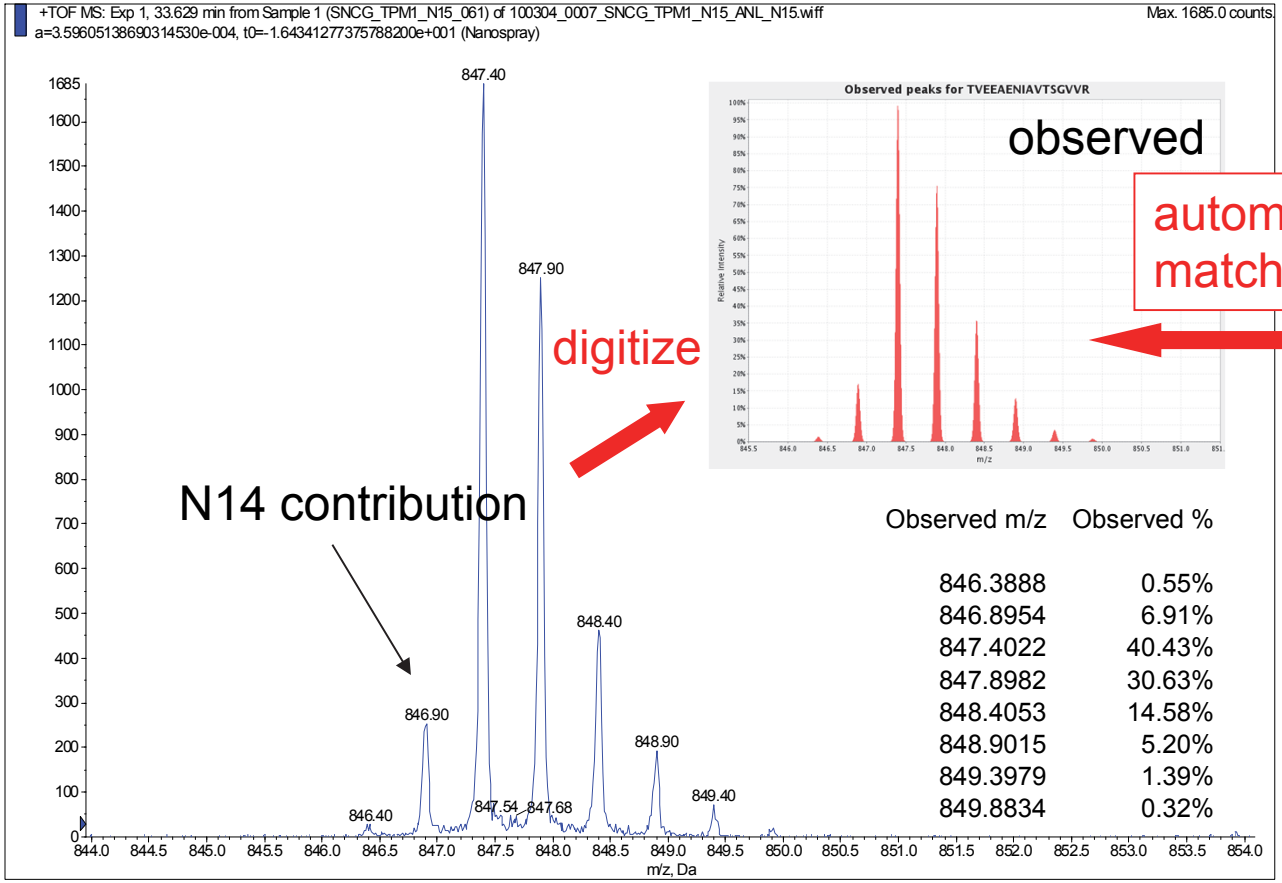
Supplemental Figure S2. Evaluation of peptide loss related to digestion and desalting and effects on CV and Determined LOD. Effect of SIS peptide spike is evaluated in terms of LOD distribution, source of peptide loss and % CV. Panel A depicts the average LOD distribution for all peptides measured at the 3 sites 56A, 56B and 65, with the SIS peptides spiked in pre- vs. post-desalt; *[note: Panel A includes peptides with LOD < 1. Seven peptides had an LOD > 1 and have been excluded]*. Panel B shows sources of peptide loss occurring during the digestion step (left side, panel B) versus desalting (right side, Panel B); see Methods for description of how calculated and Supplemental Table S8 for all underlying measurements; *[note: Panel B shows peptides with losses between 0-100%. Six peptides had losses beyond this range and have been excluded]*. Panel C illustrates the effect of pre- vs. post desalt addition of the SIS peptides on %CV.

Supplemental Figure S3

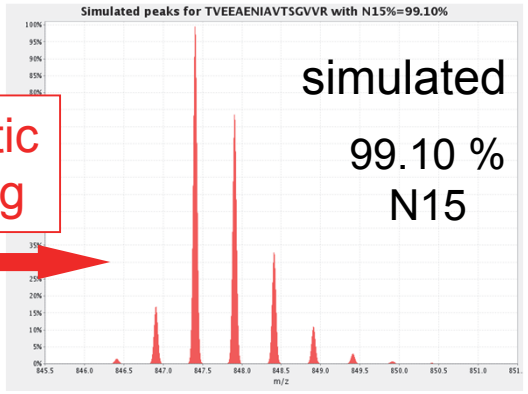


Supplemental Figure S3. Coefficient of Variation Plots for Blinded Samples in Phases II and III. In each case, the 13C/15N synthetic peptides were used as internal standards. Phase II (Panel A) shows lower %CVs at higher concentrations across all sites except Site 56B60. Phase III (Panel B) exhibits higher %CVs, due to increased sample handling at the individual sites. Site-to-site variability is more evident in the Phase III plots, as some sites have higher %CVs for the highest sample concentration.

Supplemental Figure S4. Isotopic analysis for peptide TVEEAENIAVTSGVVR



automatic matching



Observed m/z	Observed %
846.3888	0.55%
846.8954	6.91%
847.4022	40.43%
847.8982	30.63%
848.4053	14.58%
848.9015	5.20%
849.3979	1.39%
849.8834	0.32%

Simulated %	Simulated m/z
0.03%	845.91376
0.59%	846.41231
7.05%	846.91091
41.65%	847.40964
30.78%	847.91117
13.75%	848.41261
4.56%	848.91402
1.23%	849.41539
0.28%	849.91674
0.06%	850.41807
0.01%	850.91938

Determined N15 incorporation is 99.10 % for peptide TVEEAENIAVTSGVVR

Internal matching algorithm performance score:

Best Alignment Score: 0.99928. Alignment score at best match: 0.99901 (M.P. Cusack)

Supplemental Figure S4. Isotopic analysis for peptide TVEEAENIAVTSGVVR. Analysis of tryptic digestions of N15 proteins on a QSTAR Elite MS instrument to determine isotopic purity. The isotope patterns were analyzed with an in-house isotope matching program that was modified from the original program Isotope Pattern Calculator (IPC) available at PNNL (http://omics.pnl.gov/software/IPC.php).

Supplemental Table S12: Isotopic purity of U15N-labeled proteins

Protein name	N15 isotopic purity
synuclein, gamma	99.13 % N15
tropomyosin 1 (alpha)	99.13 % N15
ubiquitin-conjugating enzyme E2I (UBC9)	99.28 % N15
ubiquitin-conjugating enzyme E2C	99.03 % N15
growth factor receptor-bound protein 2	99.53 % N15
heat shock 27kDa protein 1	99.18 % N15
chloride intracellular channel 1	99.13 % N15
annexin A1	99.55 % N15
annexin A4	99.18 % N15
RAD23 homolog B	98.95 % N15
interleukin 18 (interferon-gamma-inducing factor)	99.20 % N15
lectin, galactoside-binding, soluble, 1 (galectin 1)	98.98 % N15
fascin homolog 1, actin-bundling protein	99.10 % N15
peroxiredoxin 4	98.95 % N15
peroxiredoxin 2	99.10 % N15
Aldolase C	99.13 % N15

2 tryptic peptides for each N15 labeled protein were analyzed for isotopic purity (as determined on a QSTAR Elite)