

## SUPPLEMENTAL MATERIAL FOR:

### ANALYSIS OF MUTUALLY-EXCLUSIVE ALTERNATIVELY SPLICED SERPIN-1 ISOFORMS IN *MANDUCA SEXTA* HEMOLYMPH AND IDENTIFICATION OF SERPIN-1 PROTEINASE COMPLEXES FORMED *IN VIVO*

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## SUPPLEMENTAL FIGURE LEGENDS

### **Fig. S1. Comparison of serpin-1 isoform expression in control and bacteria injected fat body.**

Relative mRNA levels of individual serpin-1 isoforms from naïve and *M. luteus* injected fat body samples were compared by t-tests with the Welch correction for variance. Error bars show standard error of the mean (SEM). Statistically significant differences ( $p$ -value < 0.05) are indicated (\*, *p*-value on graph). Other  $p$ -values are >0.05. No significant differences were seen in hemocyte samples (data not shown).

### **Fig. S2. *M. sexta* plasma separated by 2D-PAGE**

Whole *M. sexta* plasma (16  $\mu$ l) was separated by IEF with a pH range of 4.7-5.9, followed by SDS-PAGE on a 4-12% acrylamide gel and analysis by silver stain (A) or immunoblot with serpin-1 antiserum (B).

### **Fig. S3. 2D-PAGE spots selected for protease digestion.**

As in Fig. 3, *M. sexta* plasma proteins were immunoaffinity purified using serpin-1 antisera bound to protein A beads. The concentrated serpin-1 elution fractions were then separated by 2D-PAGE, pH range 4.7-5.9, and the gels stained with Coomassie blue. A) Spots excised for protease digestion using GluC in either bicarbonate buffer (*spot number followed by b*) or phosphate buffer (*spot number followed by u*) and subsequent analysis by MALDI-TOF/TOF. B) The indicated spots were analyzed by MALDI-TOF/TOF after double digestion with LysC and AspN proteases (*spot number followed by a*).

### **Fig S4. MALDI-TOF/TOF spectra from X!Tandem**

Here we provide the MALDI-TOF/TOF spectra as represented in X!Tandem for serpin-1 isoform specific peptides or protease peptides which were found in putative serpin-1-protease complexes. Mascot ion scores and X! Tandem peptide identification probabilities are listed in Supplemental Table 2.

### **Fig S5. Immunoblot detection of serpin-1B and serpin-1F in spot 5.**

2D-immunoblots of *M. sexta* plasma proteins immunoaffinity purified with serpin-1 antisera (as in Fig. 3) were probed with antisera raised against serpin-1B peptide (A) or serpin-1F peptide (B).

### **Fig. S6. MALDI-TOF spectra from trypsin digested spots 13 and 14.**

Highly similar MALDI-TOF spectra were produced following trypsin digestion of spots 13 (A) and 14 (B) (spot numbering as in Fig. 3B), showing both spots contain serpin-1E. These spectra differ in the mass of one peptide, present in spot 13 at 1592.95 and in spot 14 at 1566.95. The masses of these two peptides matches the prediction for a serpin-1 tryptic peptide from residues XXX to YYY with Tyr371 (spot 13) and His371 (spot 14).

### **Fig S7. MALDI-TOF spectra from bands 1 and 2.**

MALDI-TOF spectra from bands 1 (A) and 2 (B). Peaks corresponding to exons 1-8 of serpin-1 are labeled in blue; MO stands for oxidized methionine. Isoform-specific peaks are labeled in orange and chymotrypsin specific peaks in purple.

Table S1. Primers used for PCR analysis of serpin-1 isoforms

Location	Name	Primer sequence (5' to 3')	Product size (bp)
Exon 9A	Spn1A	CATAACACGACAAGCGAGAC	160
Exon 9B	Spn1B	TACCGGCGAGTTTGATACTA	160
Exon 9C	Spn1C	TTCTTCGTATGAACCTGTCG	161
Exon 9D	Spn1D	AAGTGTACGTCCACCCACTC	158
Exon 9E	Spn1E	AGTTATACCGCCCGTCCTA	155
Exon 9F	Spn1F	TTCATCGCTGTCGTAGACTC	192
Exon 9G	Spn1G	TTGAACCACCTGTTATCGAA	153
Exon 9H	Spn1H	CGTGGAATCCATAGACAATTT	178
Exon 9I	Spn1I	TCGGTATAGTTGCGCTATCA	179
Exon 9J	Spn1J	TGACAGACAGATGTTGTTCTGA	177
Exon 9K	Spn1K	TTCATTTTCGTGCCAAAAGT	150
Exon 9Z	Spn1Z	TTGGCATAGCATATCTGTCG	167
Exon 10	Spn1Rev	ATTCCAACCGGACGTTATT	
Exon8-9E	Spn1Efwd	GGCTGCTAACGTCATTCG	147
Exon9E-10	Spn1Erev	ATCGTTTTATGGTTGGAGA	
RPS3	RPS3Fwd	TGCGTTTCATCATGGAGT	173
	RPS3Rev	TCCTTGCCTGAGAAGTAC	

Table S2. Mass spectrometry identification of spots from 2D gels using immunoaffinity-purified serpin-1.

Trypsin spot	Protein Name	Accession Number	Iso-form <sup>1</sup>	Results from the whole protein				Results from Isoform specific peptides					
				Mascot Protein Score	Protein Score C.I.%	Pep. Count	% seq. cov.	Number by PMF (isoform)	Number by MS/MS	MS/MS Observed mass(es)	Sequence covered	Mascot ion score	X! Tandem peptide ID prob. (%)
2t	serpin 1	AAC47340.1	J	1,080	100	21	60	2 (J)	2	2422.175	337-359	173	95
										1870.942	382-397	38.5	95
3t	serpin 1	AAC47340.1	?	894	100	25	57	1 (J)	0	2422.177	337-359		
4t	serpin 1	AAC47332.1	H	1,010	100	21	57	1 (H)	1	1752.029	382-396	80	95
5t	serpin 1	AAC47333.1	?	1,010	100	19	53	1 (F)	0	1882.106	367-381		
6t	serpin 1	AAC47337.1	I	1,000	100	24	57	2 (I)	1	1543.844	370-381	45.9	95
										1618.747	382-395		
7t	serpin 1	AAC47337.1	I	896	100	19	50	2 (I)	0	1543.853	370-381		
										1618.741	382-395		
8t	serpin 1	AAC47337.1	I	1,040	100	21	56	2 (I)	2	1543.965	370-381	54	95
										1618.870	382-395	82.8	95
9t	serpin 1	AAC47342.1	A	990	100	19	56	1 (A)	1	2341.136	337-358	172	95
9t	serpin 1	AAC47338.1	Z	878	100	21	65	3 (Z)	0	2867.426	337-364		
										1792.919	365-379		
										1457.725	380-392		
10t	serpin 1	AAC47338.1	Z	995	100	20	62	3 (Z)	2	2867.447	337-364	141	95
										1792.927	365-379	74.6	95
										1457.728	380-392		
11t	serpin 1	AAC47334.1	K	1,090	100	23	64	3 (K)	2	1951.946	337-355	122	95
										1385.723	356-366		
										1468.822	367-378	57.5	95
12t	serpin 1	AAC47342.1	A	1,170	100	21	60	4 (A)	3	2341.140	337-358	166	95
										1627.940	366-378	32.5	95
										1326.721	379-390	94.4	95
										1397.760	379-391		
12t	serpin 1	AAC47335.1	E	825	100	19	55	2 (E)	0	1068.698	361-369		
										1973.994	337-355		
13t	serpin 1	AAC47335.1	E	894	100	21	54	3 (E)	2	1974.137	337-355	164	95
										1068.789	361-369	36.5	37.6
										765.571	363-369		
14t	serpin 1	AAC47335.1	E	1,000	100	21	57	4 (E)	2	1974.144	337-355	180	95
										1068.782	361-369		
										765.569	363-369		
										1566.949	370-381	48.9	95
16t	serpin 1	AAC47336.1	?	706	100	22	47	1 (G)	0	1420.689	383-396		
17t	serpin 1	AAC47335.1	E	933	100	22	54	2 (E)	0	1973.993	337-355		
										1566.826	370-381		
18t	serpin 1	AAA29336.1	?	486	100	17	39	0	0	n/a	n/a		
19t	serpin 1	AAC47340.1	J	585	100	17	45	1 (J)	1	2422.181	337-359	45.7	95
19t	serpin 1	AAC47342.1	?	453	100	17		1 (A)	0	2341.144	337-358		
20t	serpin 1	AAC47342.1	?	676	100	19	48	1 (A)	0	2341.142	337-358		
21t	serpin 1	P14754.1	?	126	100	11	24	0	0	n/a	n/a		
22t	serpin 1	AAC47335.1	?	263	100	19	42	1 (E)	0	1974.003	337-355		
23t	serpin 1	AAC47335.1	?	223	100	13	31	1 (E)	0	1973.987	337-355		
24t	serpin 1	AAC47335.1	E	717	100	24	50	3 (E)	1	1973.996	337-355	50.6	95
										1068.696	361-369		
										765.512	363-369		
25t	serpin 1	AAC47335.1	E	540	100	23	50	3 (E)	1	1973.997	337-355	20.6	82.4
										1068.695	361-369		

26t	PPO1	O44249.3	n/a	382	100	24	33	n/a	3	1566.830	370-381	65.7	95
										1352.673	546-557	43.7	95
										1516.767	408-419	32.1	95
26t	PPO2	Q25519.3	n/a	118	100	16	24	n/a		783.4206	289-295		
										n/a	n/a		

LysC/ AspN Spot	Protein Name	Accession Number	Iso-form <sup>1</sup>	Results from the whole protein				Isoform specific peptides					
				Mascot Protein Score	Protein Score C.I.%	Pep. Count	% seq. cov.	Number by PMF (isoform)	Number by MS/MS	MS/MS Observed mass(es)	Sequence covered	Mascot ion score	X! Tandem peptide ID prob. (%)
2	serpin 1	1K9O_I	?	133	100	11	35	2(B)	0	1107.584	365-374		
										1214.620	356-364		
2	serpin 1	AAC47333.1		131	100	11		2 (F)		1183.683	373-381		
										2875.441	373-397		
3	serpin 1	AAC47332.1	H	120	100	14	41	3 (H)	0	1412.795	371-381		
										1566.885	384-396		
										2475.286	362-381		
3	serpin 1	AAC47337.1		112	99.997	13	39	2 (F)		1183.691	373-381		
										2875.437	373-397		
4	serpin 1	AAC47332.1	H	482	100	16	53	3 (H)	2	1412.786	371-381	55.5	95
										1566.856	384-396	81.8	95
										2475.296	362-381		
5	serpin 1	P14754.1	B	404	100	16	49	2 (B)	1	1214.674	370-378	41.8	94
										1091.599	379-388		
										1107.593	379-388		
5	serpin 1	AAC47333.1	F	380	100	16	50	2 (F)		1377.743	362-372		
										1183.688	373-381		
6	serpin 1	AAC47337.1	?	272	100	13	53	1 (I)	0	3143.615	370-395		
7	serpin 1	AAC47333.1	F	405	100	18	51	2 (F)	0	1183.679	373-381		
										1694.944	382-397		
										1726.879	382-397		
7	serpin 1	AAC47337.1	I	394	100	16	51	1 (I)		3143.647	370-395		
8	serpin 1	AAC47341.1	D	350	100	17	52	2 (D)	0	1366.757	384-395		
										1431.789	373-383		
9	serpin 1	AAC47337.1	?	200	100	17	56	1(I)	0	3086.495	370-395		
10	serpin 1	AAC47338.1	Z	414	100	14	43	2 (Z)	1	1305.732	371-380	48.8	95
										1086.577	383-392		
11	serpin 1	AAC47334.1	K	278	100	14	44	2 (K)	0	1385.760	356-366		
										1658.795	379-392		
										1674.790	379-392		
12	serpin 1	AAC47333.1		284	100	16	48	2 (F)	0	2206.092	337-358		
										2875.440	373-397		
13	serpin 1	AAC47335.1	E	463	100	15	47	4 (E)	1	2300.253	337-358		
										1068.707	361-369	35.5	62.9
										1566.876	370-381		
										1183.659	373-381		
14	serpin 1	AAC47335.1	E	517	100	17	49	5(E)	3	2300.239	337-358	61.5	95
										1068.707	361-369	37.1	73.6
										1566.851	370-381		
										1183.644	373-381	33.6	56.7
										1368.716	373-383		
18	HP8	AAV91006.1	n/a							1458.794	124-134	27.2	70
19	HP8	AAV91006.1	n/a	81	95.862	6	24	n/a	1	1458.794	124-134	51.6	95
20	HP8	AAV91006.1	n/a					n/a	1	1458.789	124-134	56.3	95

Results from the whole protein								Isoform specific peptides					
GluC Bicarbonate Spot	Protein Name	Accession Number	Iso-form <sup>1</sup>	Mascot Protein Score	Protein Score C.I.%	Pep. Count	% seq. cov.	Number by PMF (isoform)	Number by MS/MS	MS/MS Observed mass(es)	Sequence covered	Mascot ion score	X! Tandem peptide ID prob. (%)
2b	serpin 1	AAC47340.1	J	159	100	9	25	1 (J)	1	1526.845	385-397	79.8	95
3b	serpin 1	AAC47343.1		107	99.995	9	27	1 (B)	0	1322.708	367-376		
4b	serpin 1	AAC47343.1	B	154	100	10	33	2 (B)	0	1322.708	367-376		
										1786.955	377-392		
5b	serpin 1	AAC47343.1	B	146	100	10	36	2 (B)	1	1322.703	367-376	31.5	95
										1771.059	377-392		
										1786.969	377-392		
5b	serpin 1	AAC47337.1	I	109	99.997	10	33	2 (I)	0	948.530	354-362		
										1538.853	354-367		
7b	serpin 1	1K9O_I	?	124	100	8	23	0	0	n/a	n/a		
8b	serpin 1	1K9O_I	?	105	99.993	7	18	0	0	n/a	n/a		
9b	serpin 1	1K9O_I	?	120	100	11	32	0	0	n/a	n/a		
11b	serpin 1	1K9O_I	?	88	99.625	8	23	0	0	n/a	n/a		
14b	serpin 1	1K9O_I	?	116	100	10	31	0	0	n/a	n/a		
19b	serpin 1	1SEK_A	?	123	100	10	35	0	0	n/a	n/a		
19b	HP8	AAV91006.1	n/a						1	1334.69	260-271	43.6	95

Results from the whole protein								Isoform specific peptides					
GluC Phosphate Spot	Protein Name	Accession Number	Iso-form <sup>1</sup>	Mascot Protein Score	Protein Score C.I.%	Pep. Count	% seq. cov.	Number by PMF (isoform)	Number by MS/MS	MS/MS Observed mass(es)	Sequence covered	Mascot ion score	X! Tandem peptide ID prob. (%)
4p	serpin 1	AAC47332.1	H	142	100	15	44	2 (H)	1	1692.987	385-398		
										1859.991	370-384	33.5	95
4p	serpin 1	AAC47343.1	B	113	99.999	14	40	1 (B)	0	1322.705	367-376		
5p	serpin 1	AAC47343.1	B	82	98.472	10	29	2 (B)	1	1322.700	367-376	32.6	78
										1771.046	377-392		
										1786.985	377-392		
7p	serpin 1	AAC47336.1	G	115	100	13	38	1 (G)	0	3024.661	371-396		
8p	serpin 1	AAC47337.1	I	201	100	14	42	2 (I)	0	948.539	354-362		
										3384.889	368-395		
11p	serpin 1	AAC47334.1	?	113	99.999	12	35	1 (K)	0	2358.320	348-368		
12p	serpin 1	AAC47342.1	?	219	100	11	24	0	0	n/a	n/a		
13p	serpin 1	AAC47342.1	?	201	100	14	40	0	0	n/a	n/a		
14p	serpin 1	AAC47339.1	?	150	100	14	36	2 (C)	0	1137.639	348-358		
										1488.709	359-371		

<sup>1</sup>Isoform identification based on the presence of at least two isoform specific peptides by PMF or at least one isoform specific peptide confirmed by MS/MS with an X!Tandem peptide identification probability of 95%

Figure S1

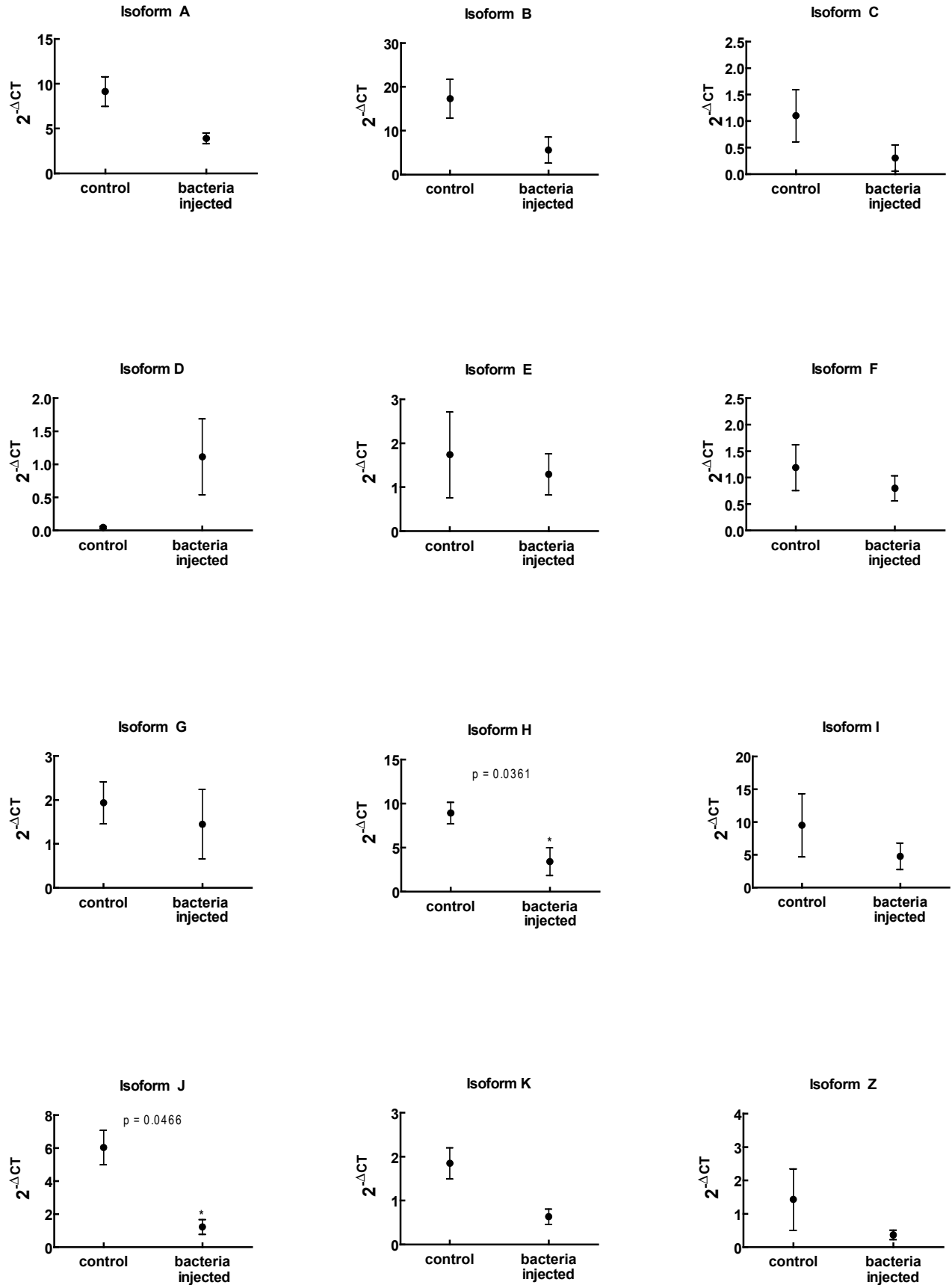


Figure S2

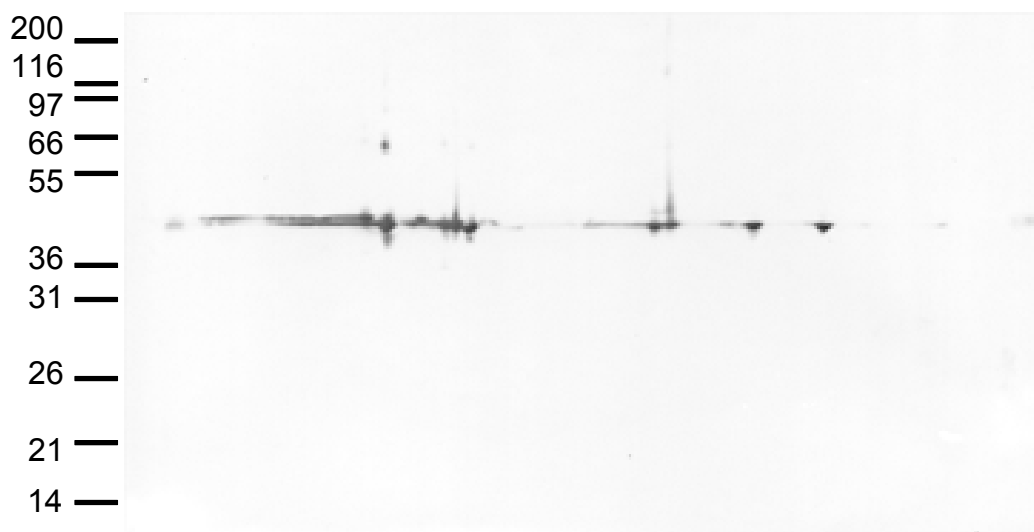
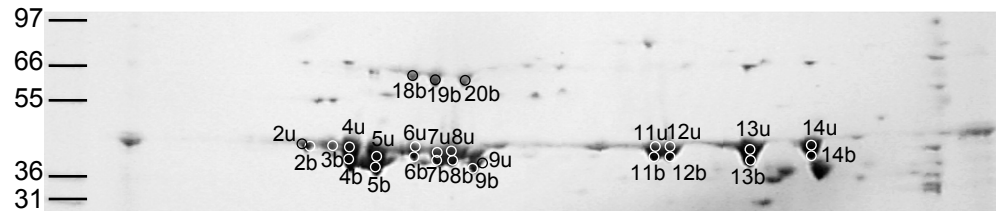


Figure S3

A.



B.

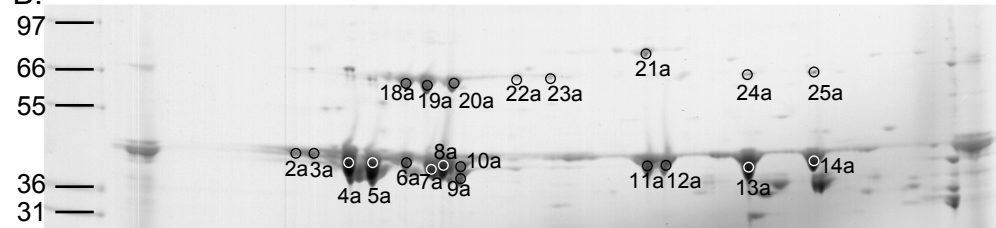
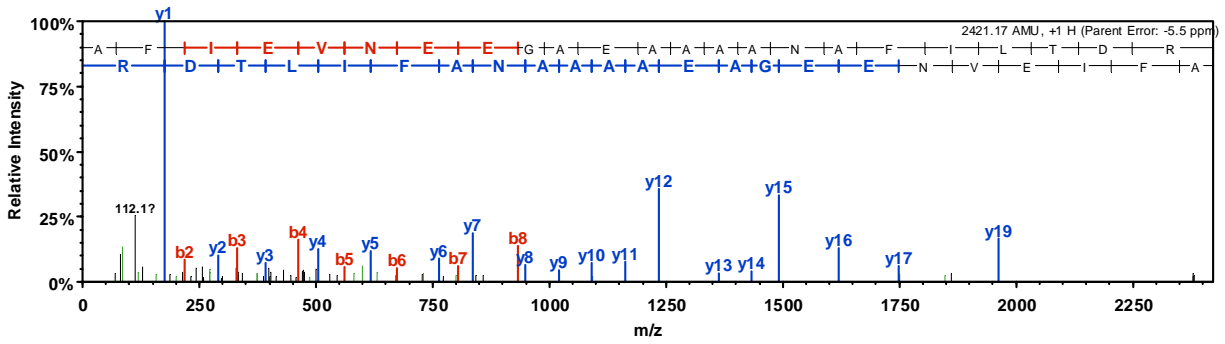


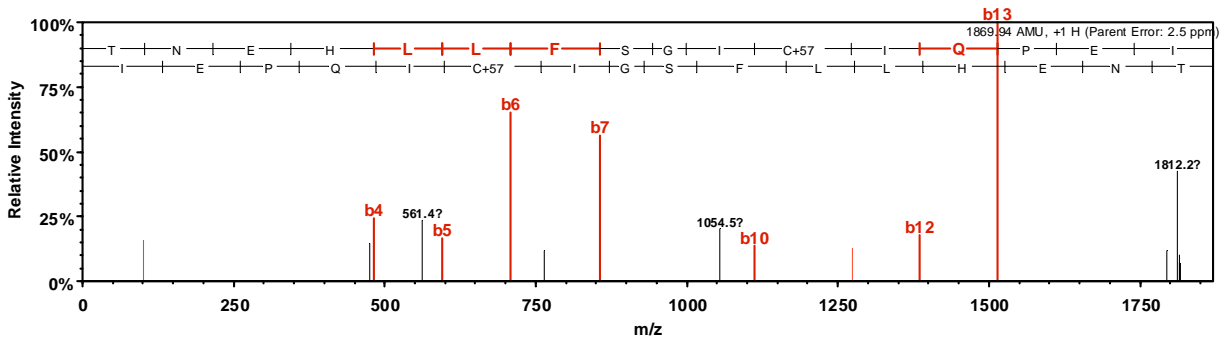


Figure S4

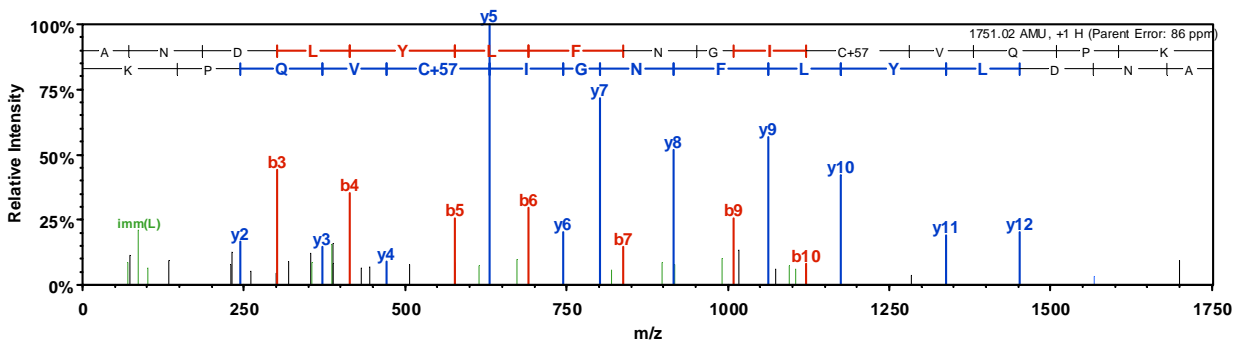
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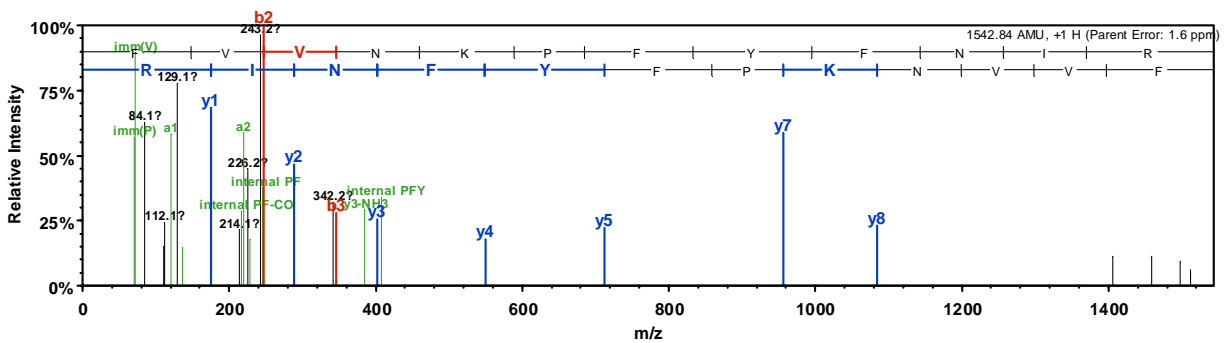
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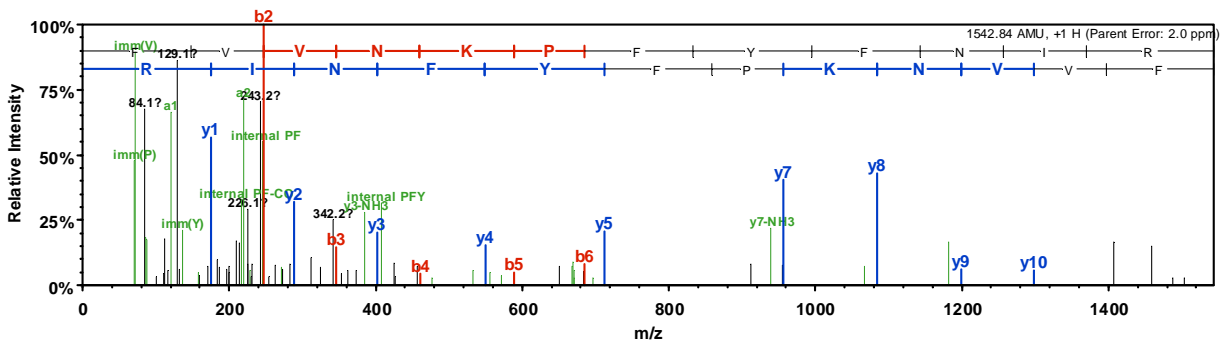
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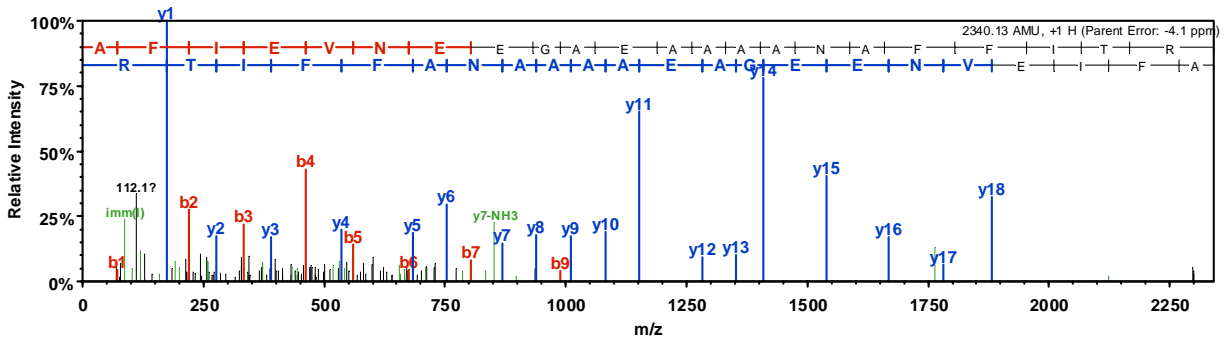
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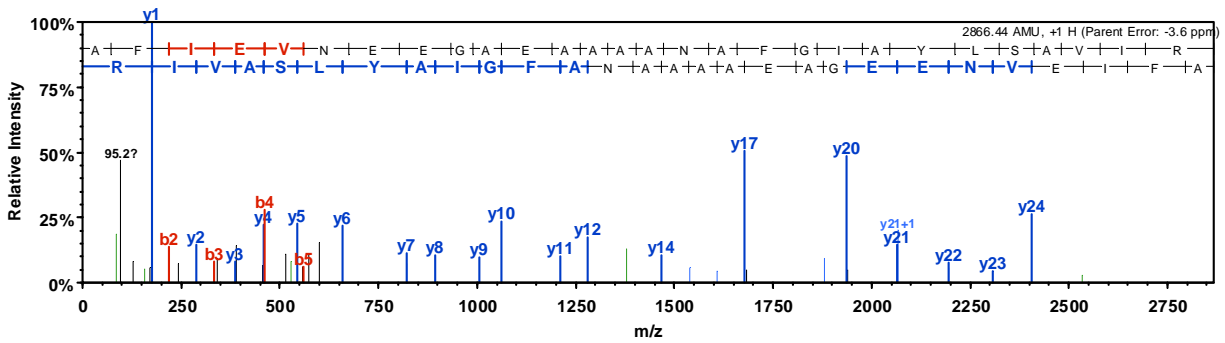
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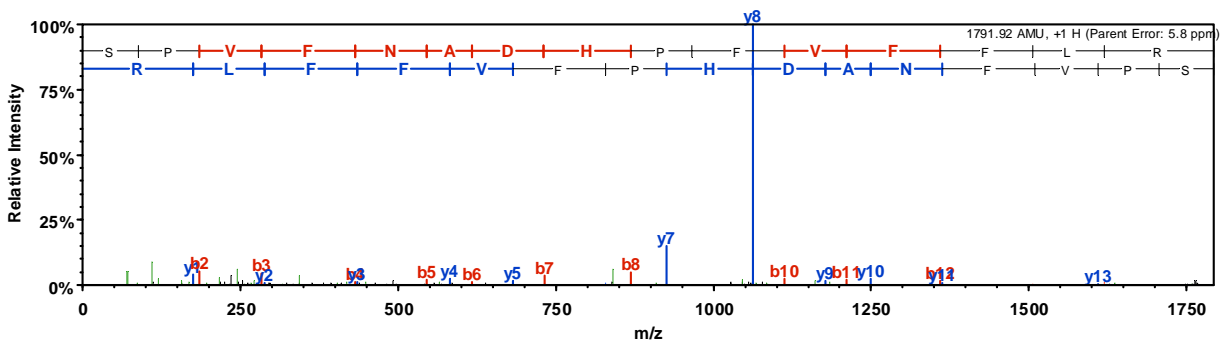
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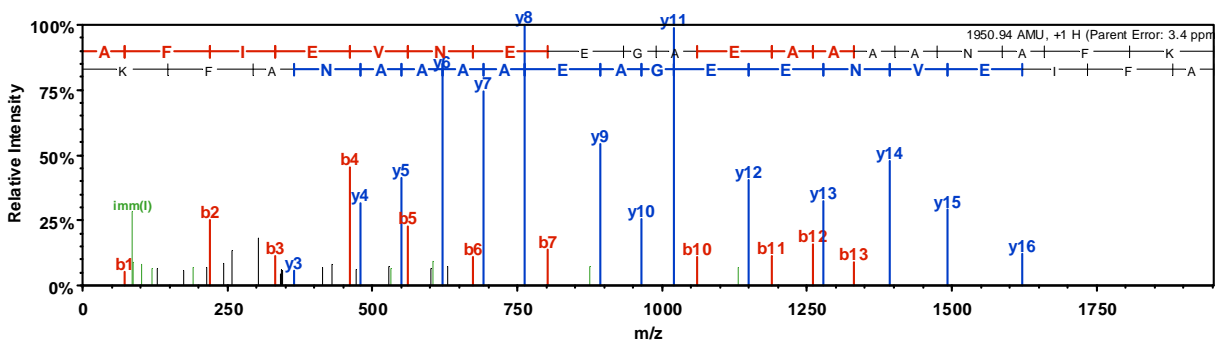
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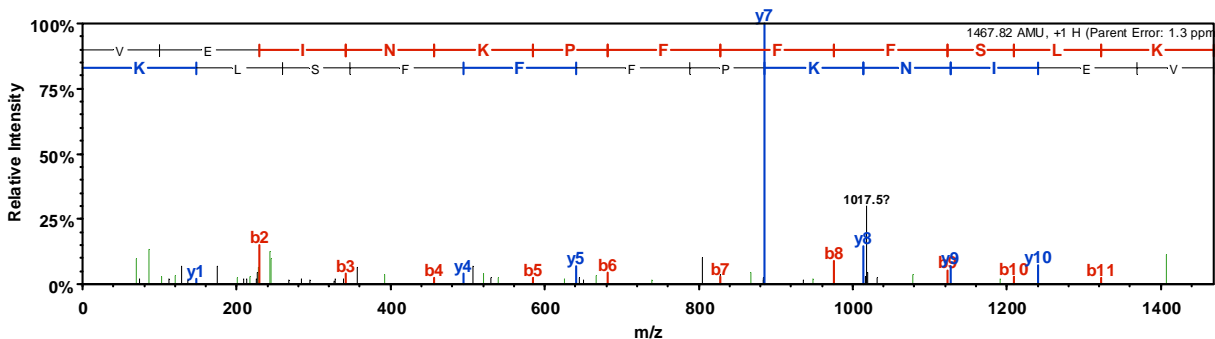
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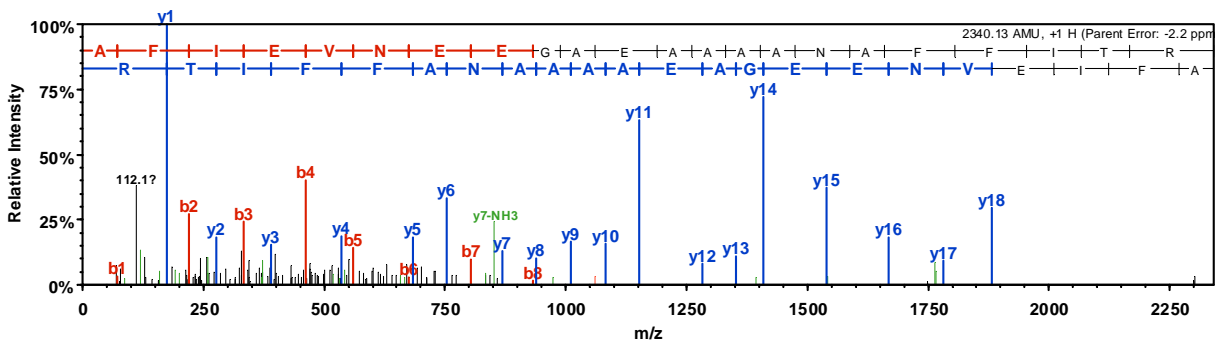
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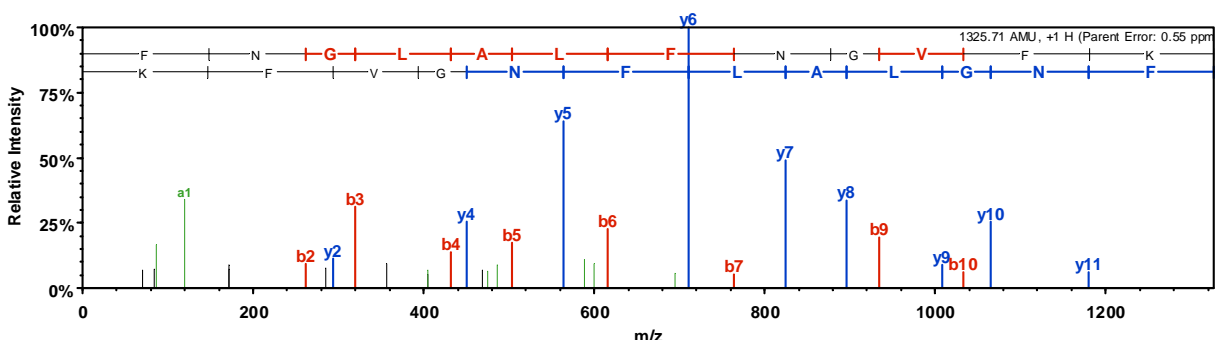
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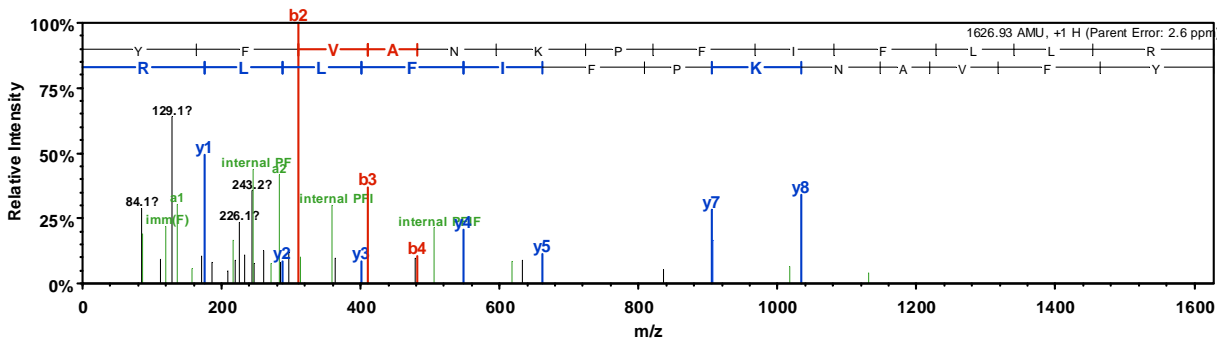
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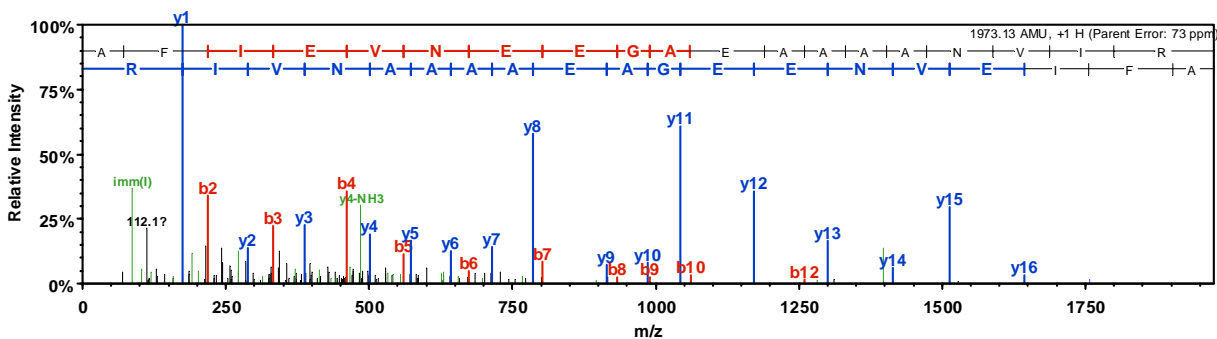
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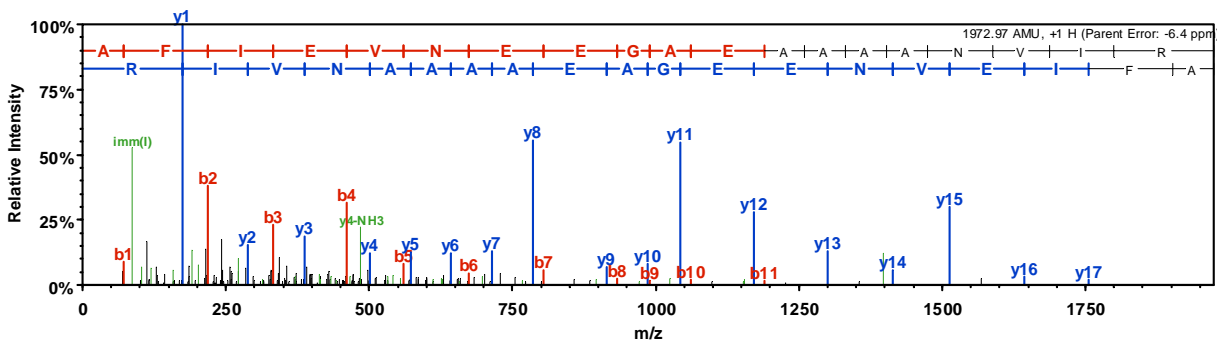
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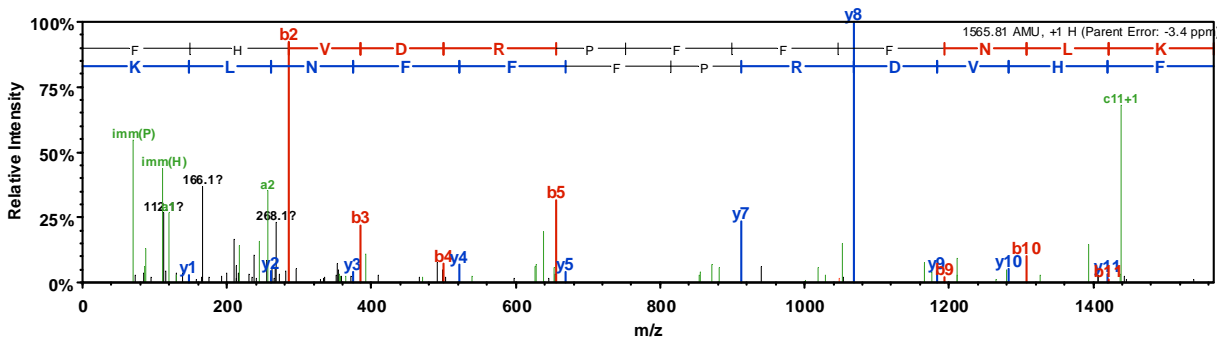
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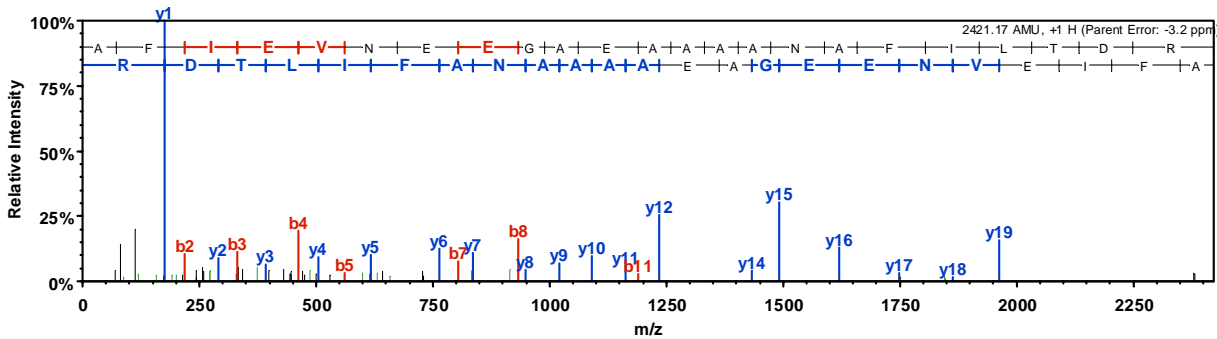
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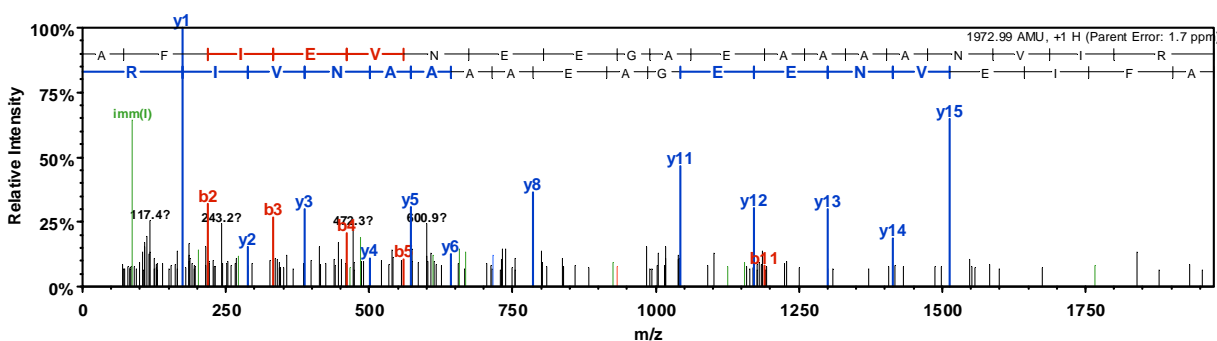
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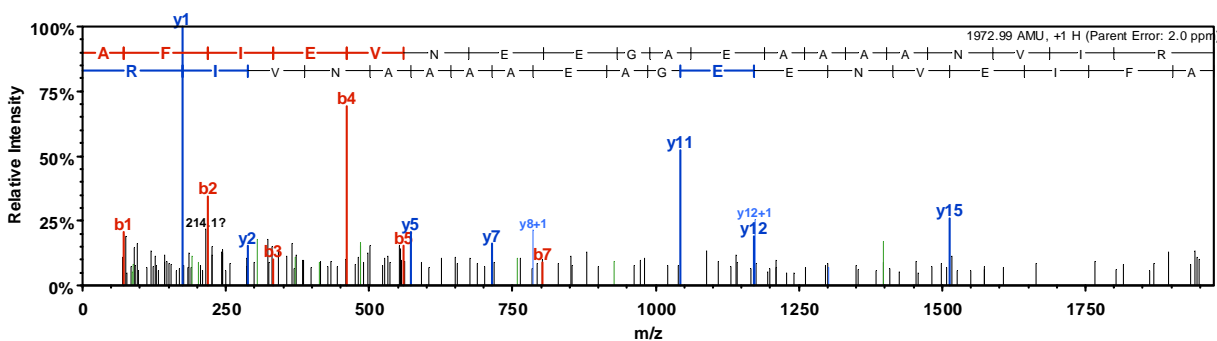
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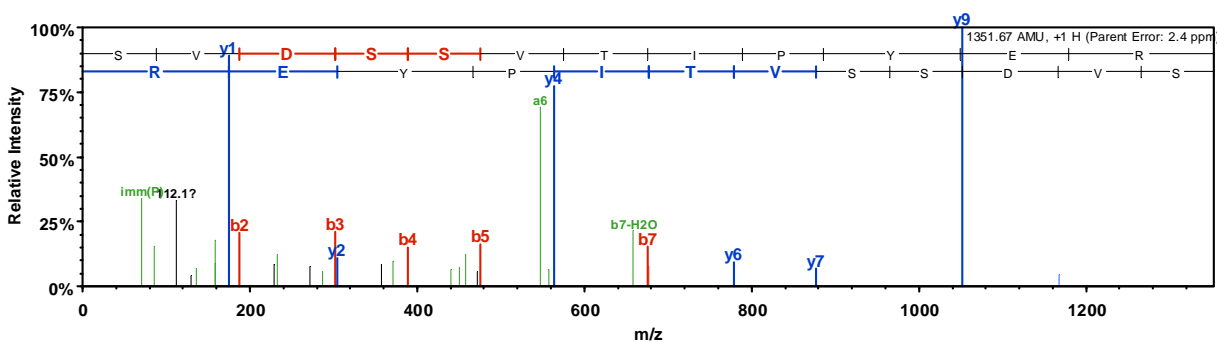
24\_trypsin Serpin-1E



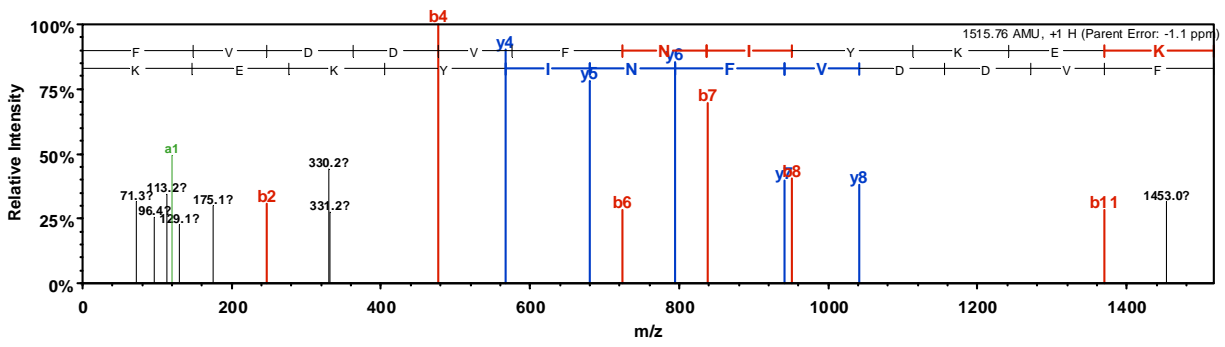
25\_trypsin Serpin-1E



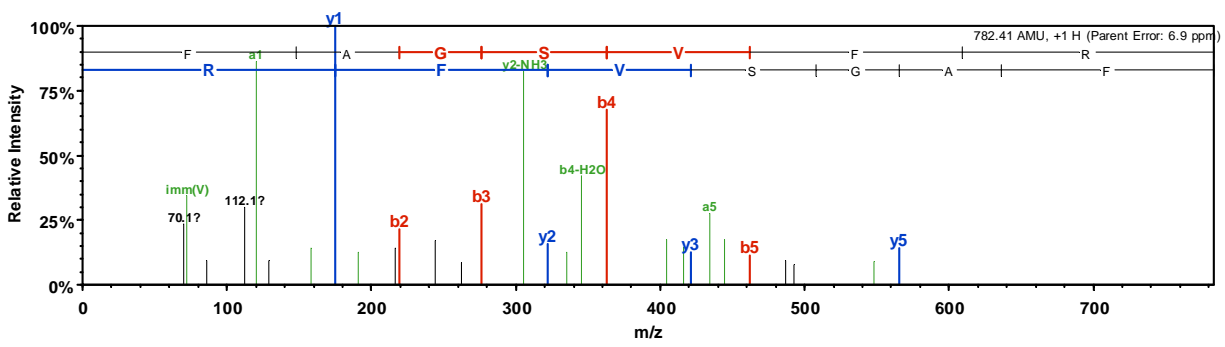
26\_trypsin Prophenoloxidase-1 peptide 1



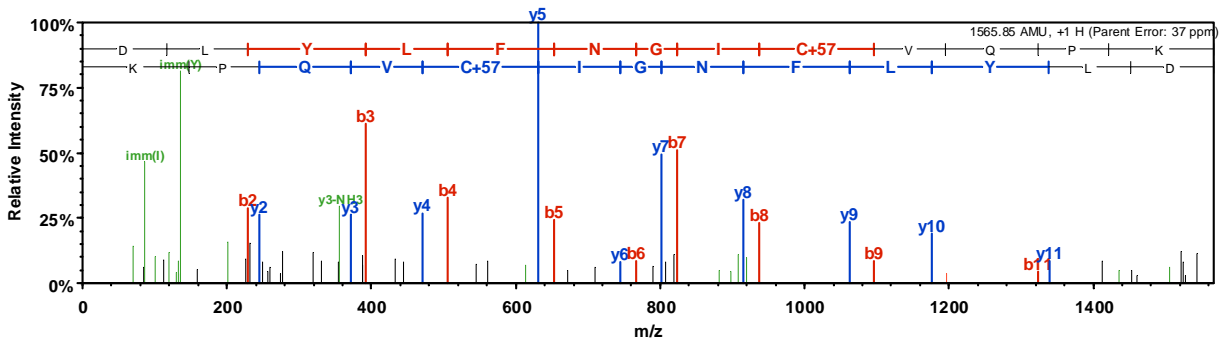
26\_trypsin Prophenoloxidase-1 peptide 2



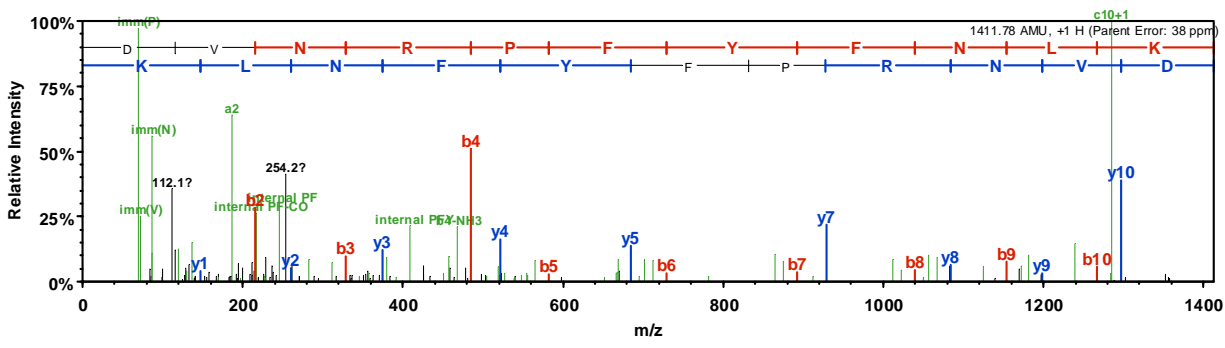
26\_trypsin Prophenoloxidase-1 peptide 3



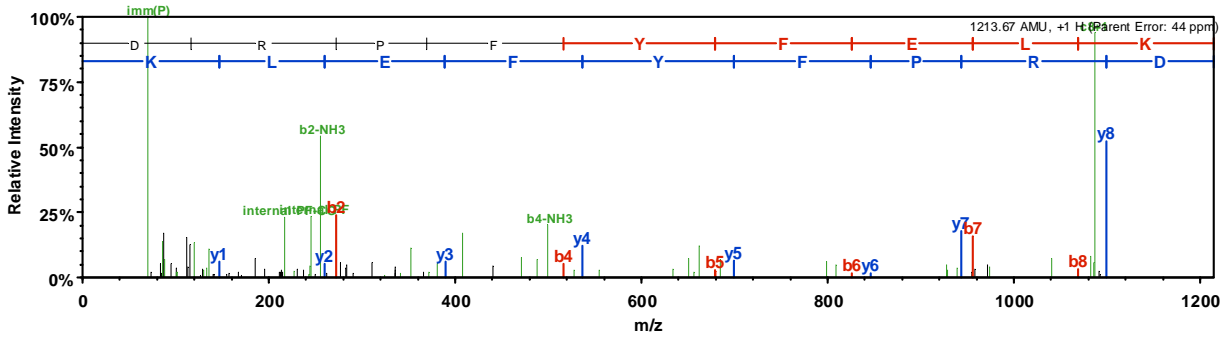
4\_LysC/AspN Serpin-1H peptide 1



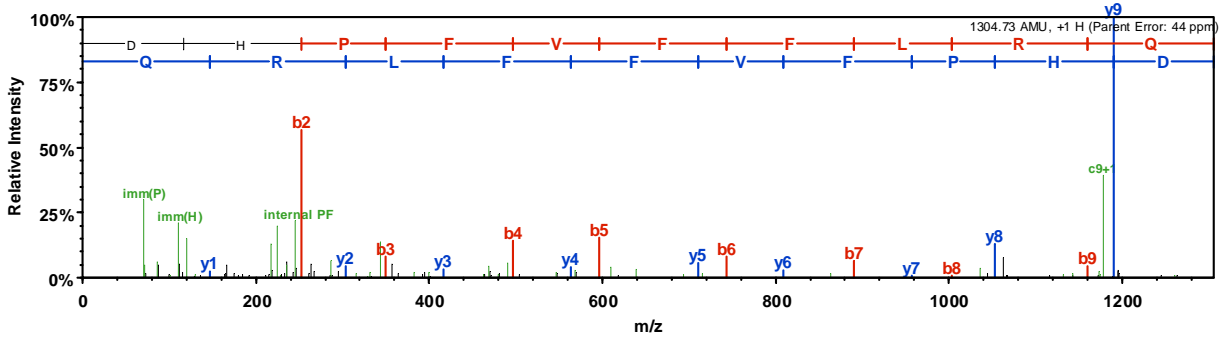
4\_LysC/AspN Serpin-1H peptide 2



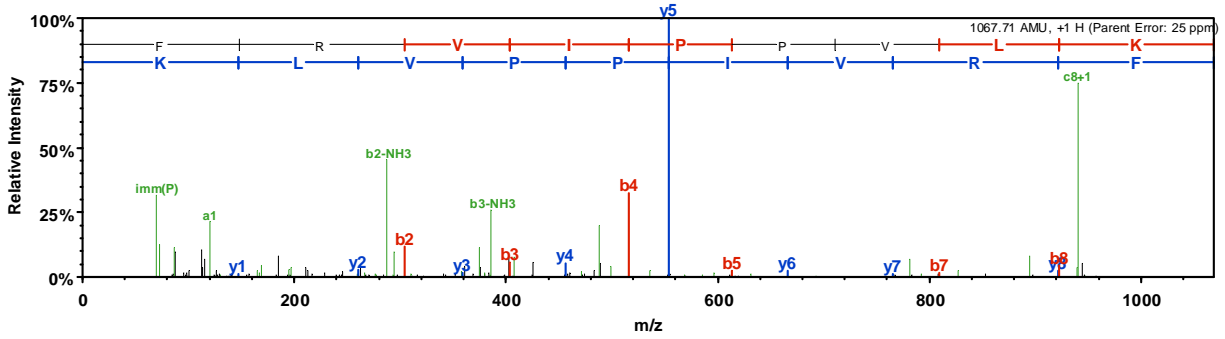
5\_ LysC/AspN Serpin-1B



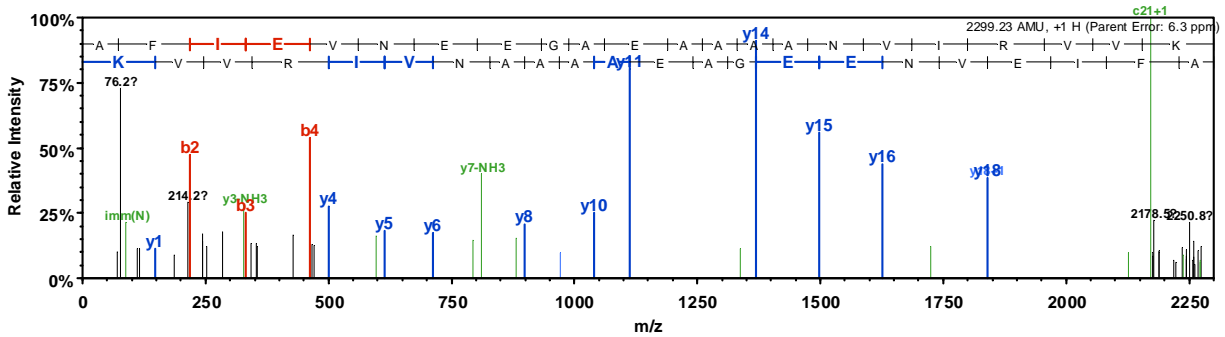
10\_ LysC/AspN Serpin-1Z



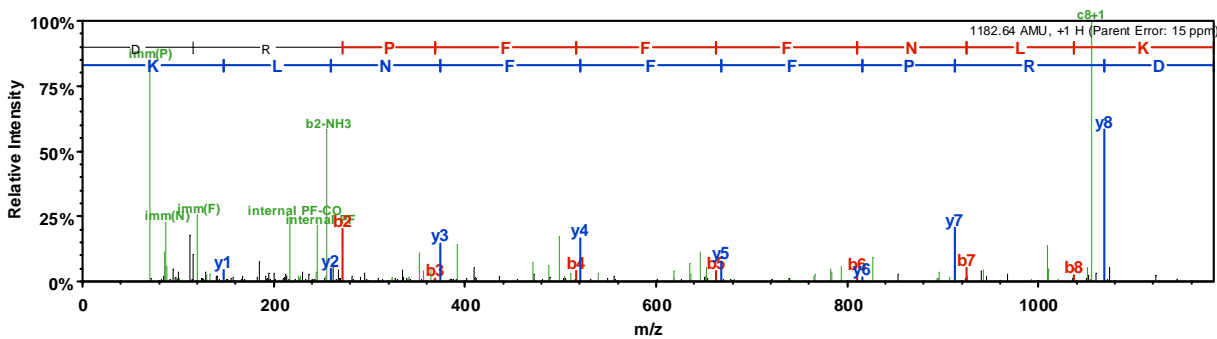
13\_ LysC/AspN Serpin-1E



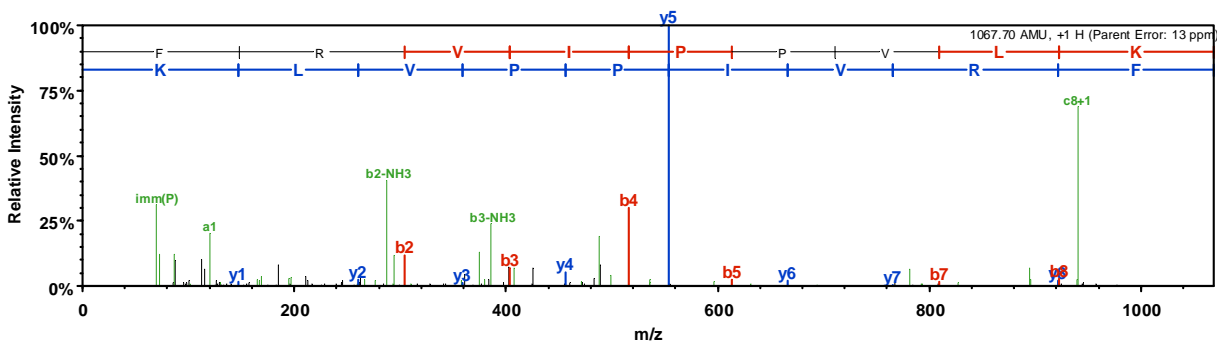
14\_ LysC/AspN Serpin-1E peptide 1



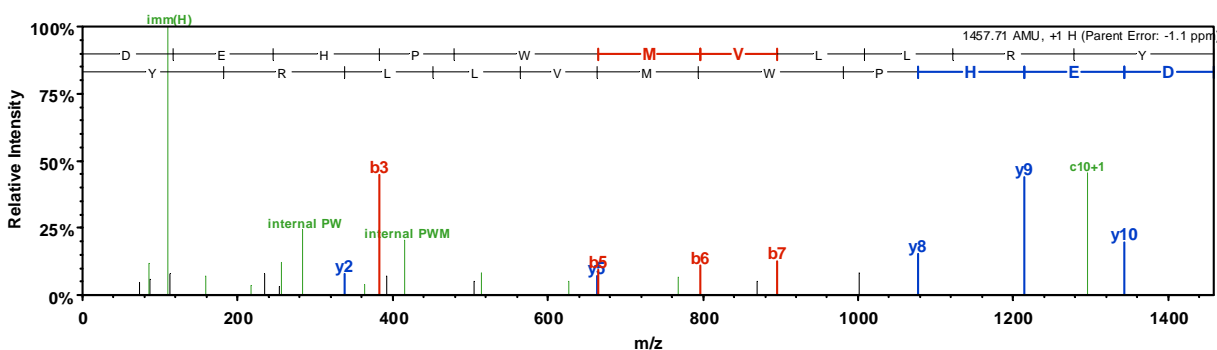
14\_ LysC/AspN Serpin-1E peptide 2



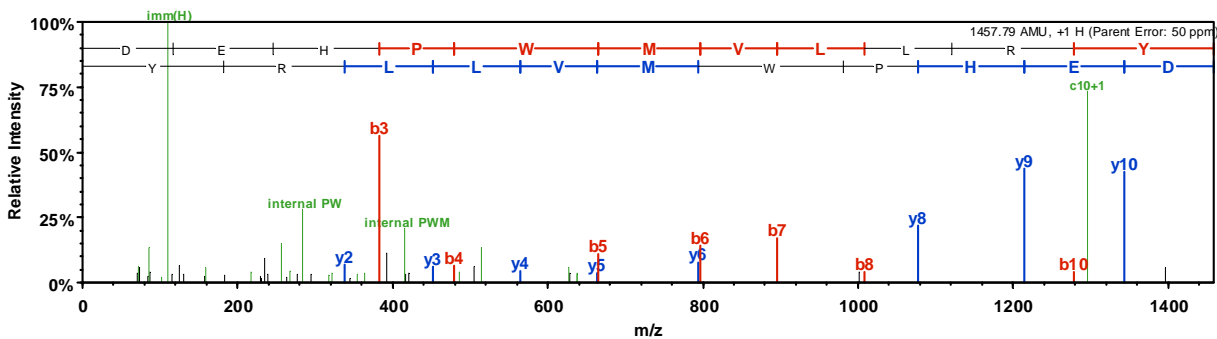
14\_ LysC/AspN Serpin-1E peptide 3



18\_ LysC/AspN HP 8

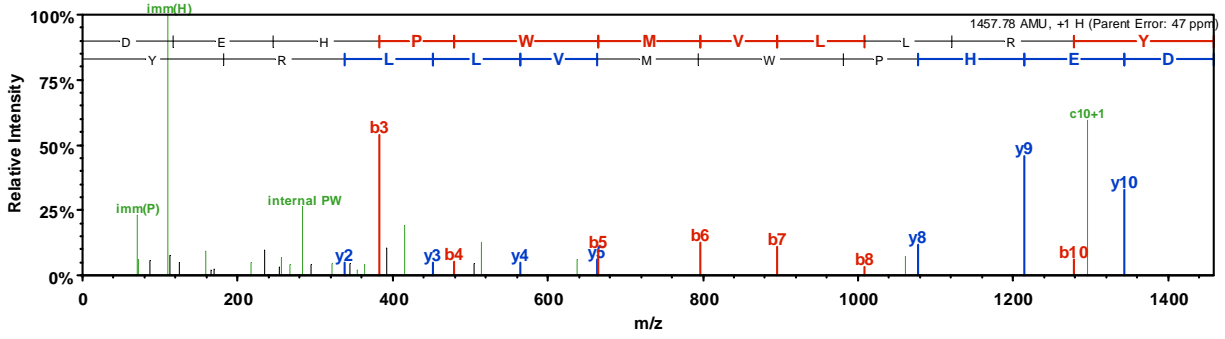


19\_ LysC/AspN HP 8

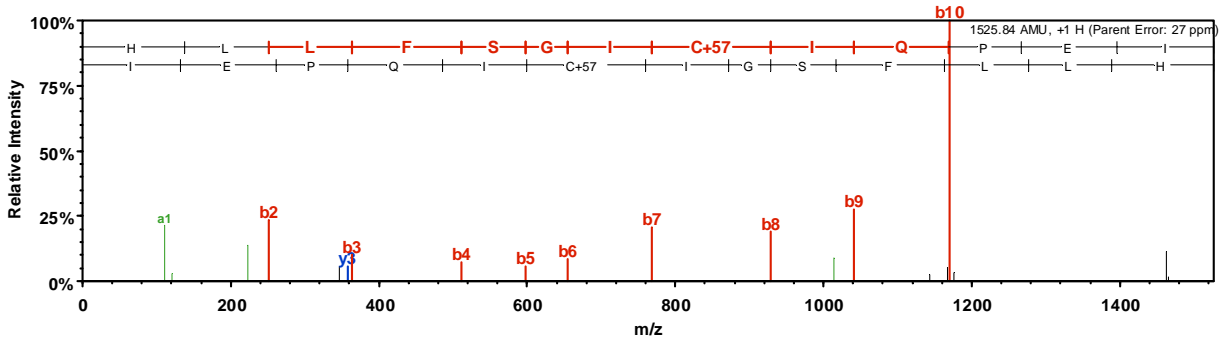




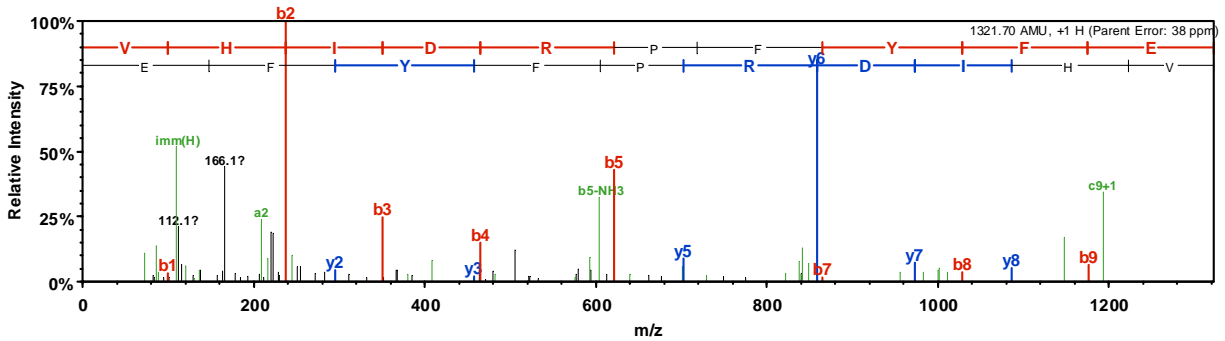
20\_ LysC/AspN C HP 8



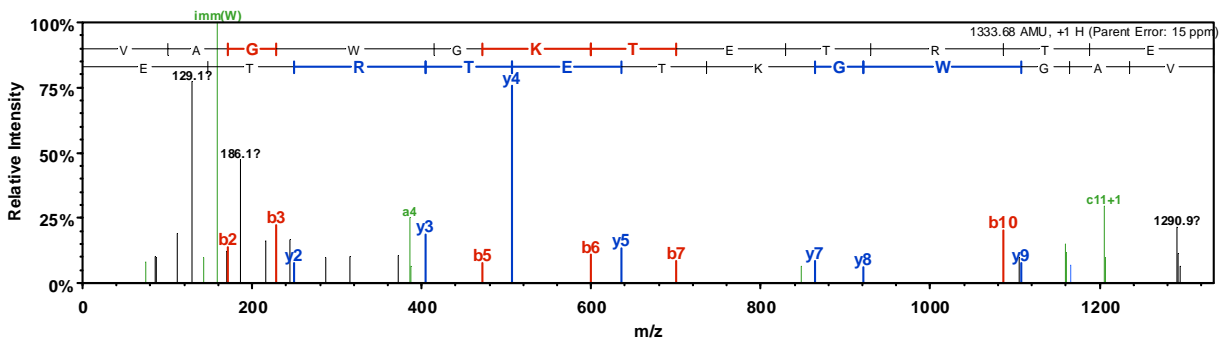
2b\_ Glu-C Serpin-1J



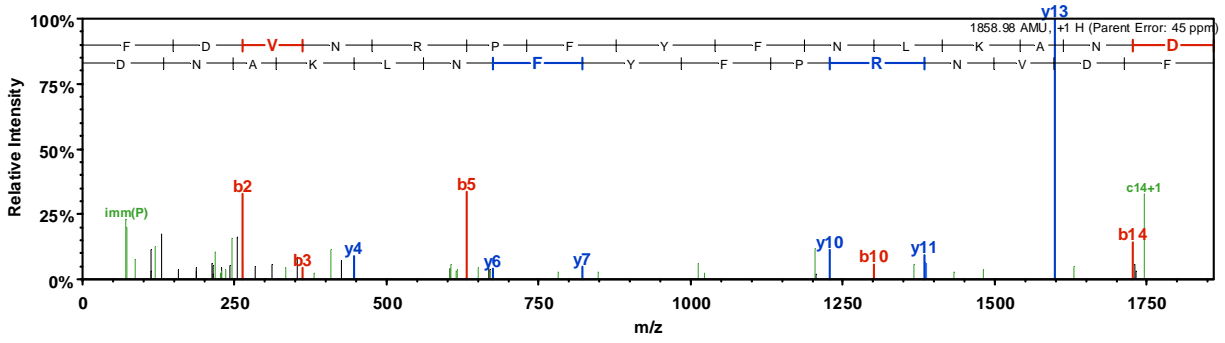
5b\_ Glu-C Serpin-1B



19b\_ Glu-C HP8



### 4p\_Glu-C Serpin-1H



### 5p\_GluC Serpin-1B

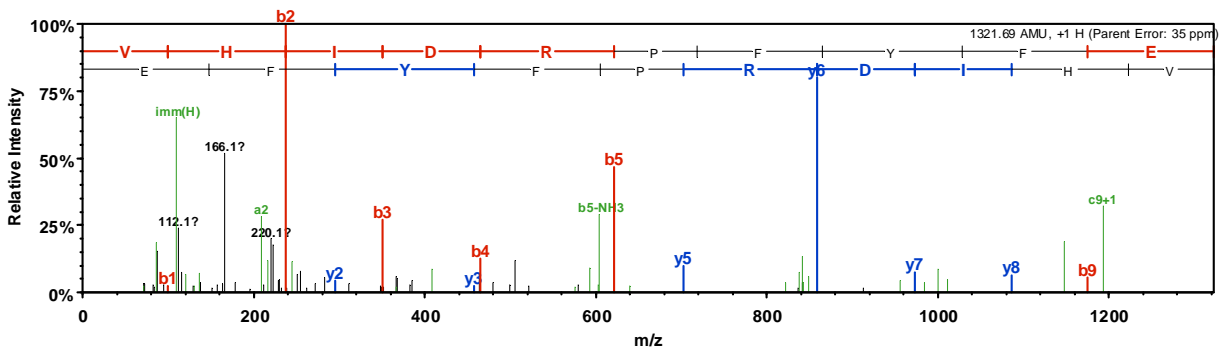


Figure S5

A. Serpin-1B antibody



B. Serpin-1F antibody

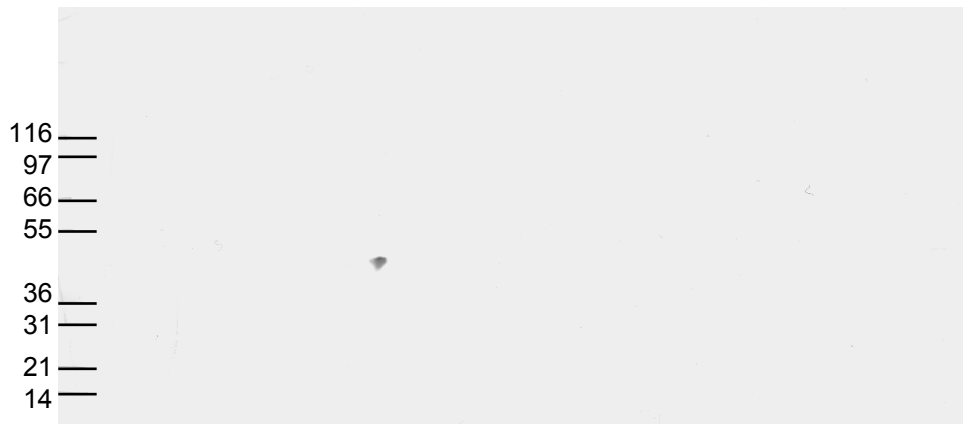
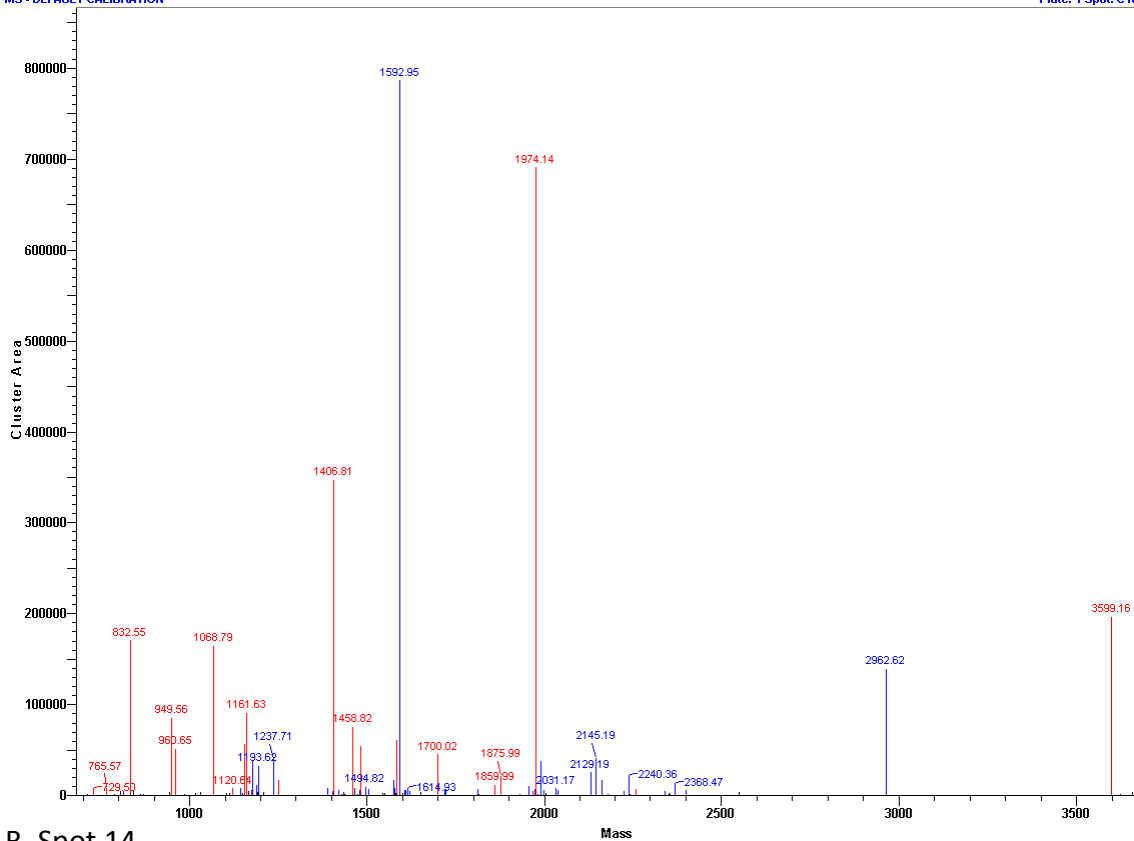


Figure S6

A. Spot 13

MS - DEFAULT CALIBRATION

Plate: 1 Spot: C10



B. Spot 14

MS - DEFAULT CALIBRATION

Plate: 1 Spot: C11

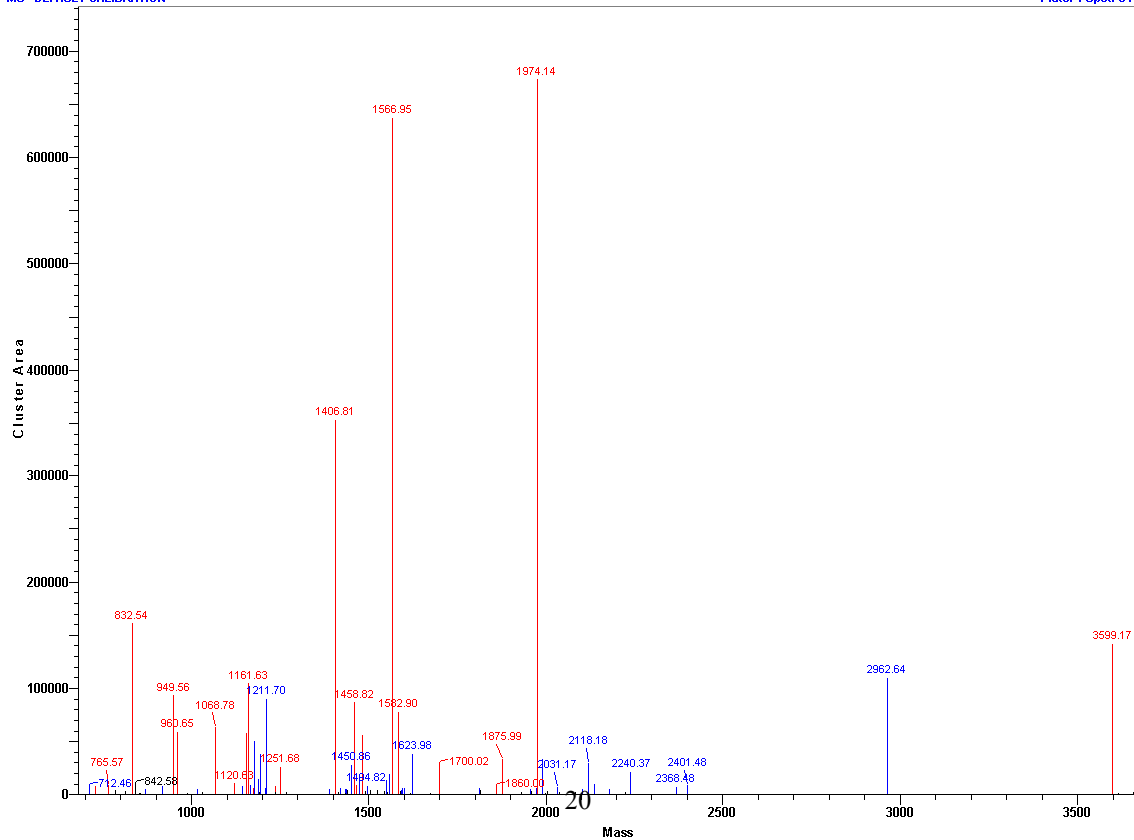
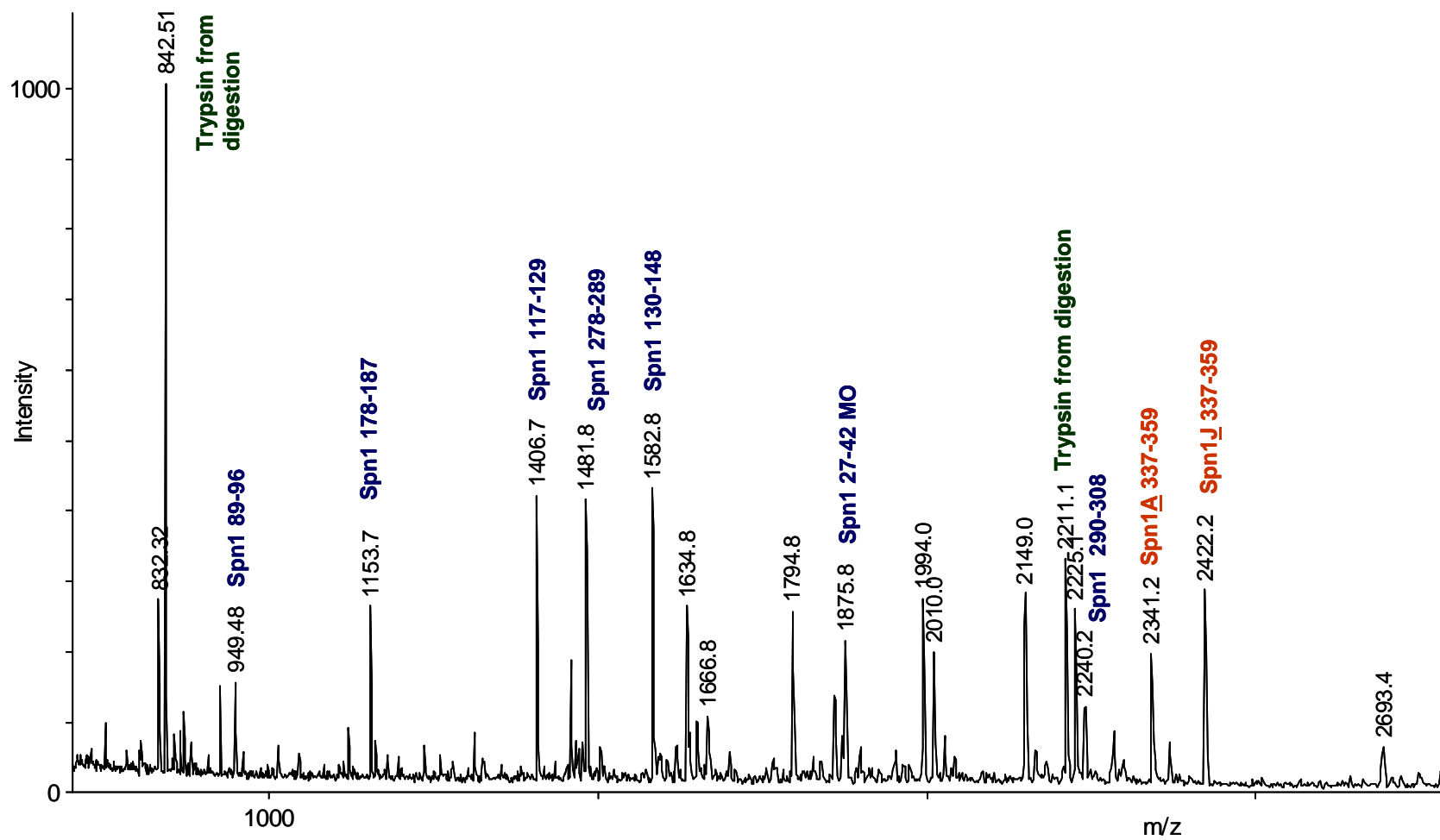


Figure S7

A.



**B**