Supporting information

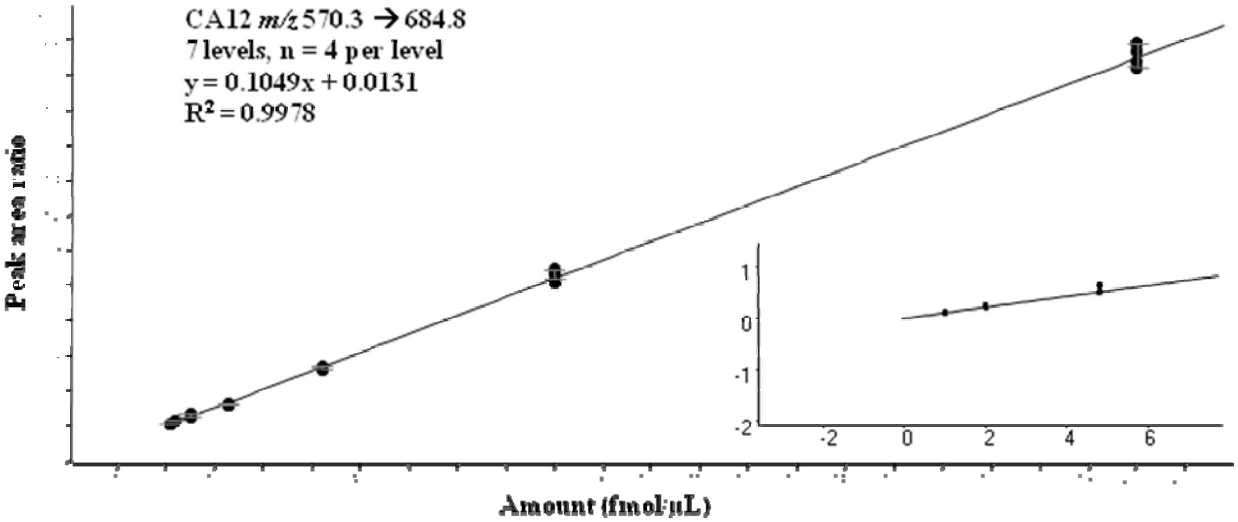
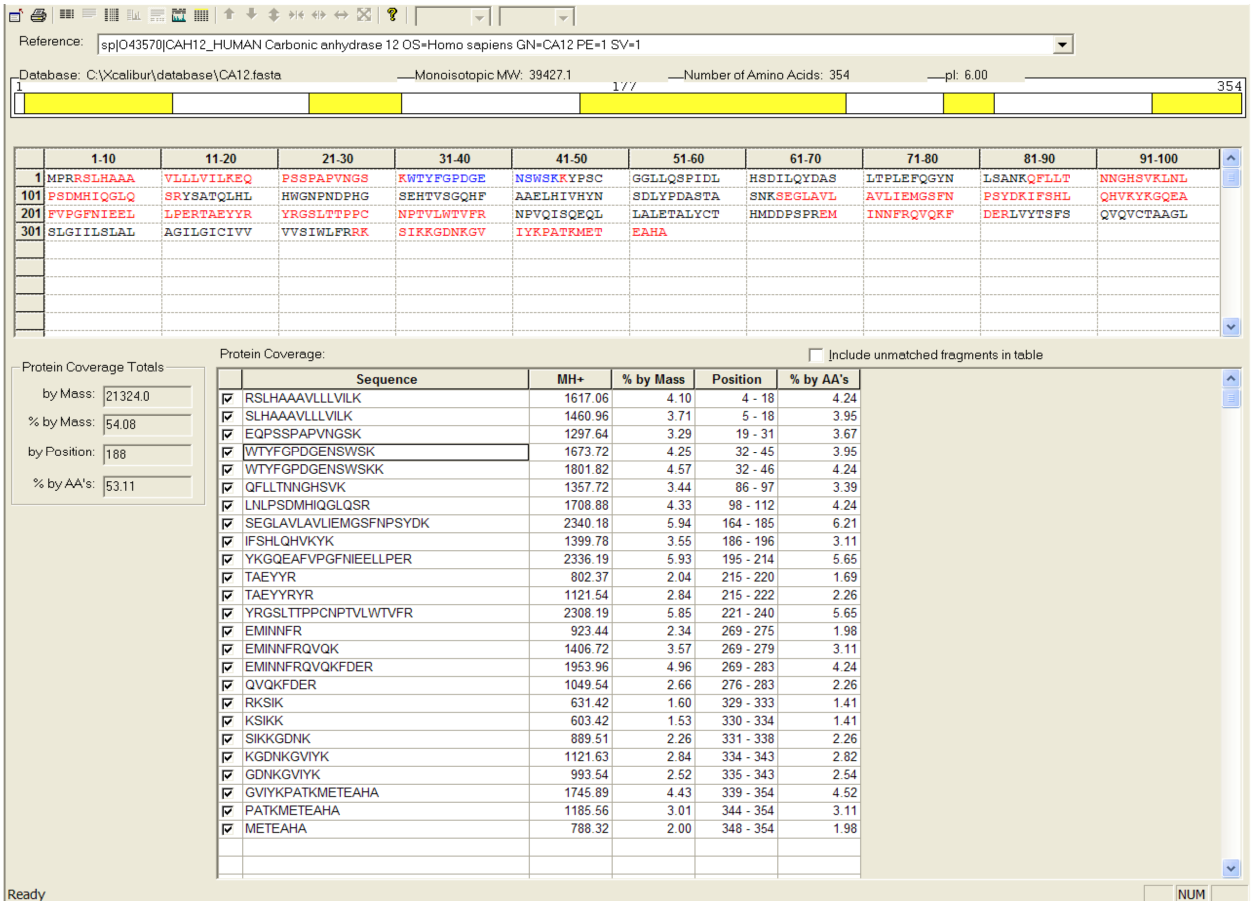


Figure S-2. Calibration curve for quantitation of enriched CA12 peptide 1 *m/z* 570.3→ 684.8. The peak area ratios of light peptide:heavy peptide for different amounts of light peptide spiked into pRC3 blank matrix was measured using LC-SRM and plotted versus amount of light peptide. The inset is the enlarged region from 0.8 to 5.1 fmol/µL of the unlabeled peptide.

Data-base results for purified and trypsin digested CA12-FLAG protein standard. For this, Sequest algorithm incorporated into the Bioworks software, version 3.1, SR1.4 (Thermo Electron, San Jose, CA) was used. Search parameters included carbamidomethylation of cysteines, ± 1.4 Da and ± 1.0 Da tolerance for precursor and product ion masses, respectively. Only peptides resulting from tryptic cleavages with up to one missed cleavage were searched. The Sequest results were filtered by correlation score (Xcorr) values selected to obtain highly confident peptide and protein identifications: Xcorr 1.9, 2.2, and 3.75 for singly, doubly, and triply charged peptide ions, respectively.



Reference: sp|O43570|CAH12_HUMAN Carbonic anhydrase 12 OS=Homo sapiens GN=CA12 PE=1 SV=1

Database: C:\Xcalibur\database\CA12.fasta

Monoisotopic MW: 39427.1

Number of Amino Acids: 354

pI: 6.00

	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
1	MPRRSLHAAA	VLLLVILKEQ	PSSPAPVNGS	KWTYFGPDGE	NSWSKKYPSC	GGLLQSPIDL	HSDILQYDAS	LTPLEFQGYN	LSANKQFLLT	NNGHSVKLNL
101	PSDMHIQGLQ	SRYSATQLHL	HWGNPNDPHG	SEHTVSGQHF	AAELHIVHYN	SDLYPDASTA	SNKSEGLAVL	AVLIEMGSFN	PSYDKIFSHL	QHVKYKGQEA
201	FVPGFNIEEL	LPERTAEYYR	YRGSLTTPPC	NPTVLWTVFR	NPVQISQEQL	LALETALYCT	HMDDPSPREM	INNFRQVQKF	DERLVYTSFS	QVQVCTAAGL
301	SLGIILSLAL	AGILGICIVV	VVSIWLFRRK	SIKKGDNKGV	IYKPATKMET	EAHA				

Protein Coverage Totals

by Mass: 21324.0

% by Mass: 54.08

by Position: 188

% by AA's: 53.11

Protein Coverage:

☐ Include unmatched fragments in table

	Sequence	MH+	% by Mass	Position	% by AA's
☑	RSLHAAAVLLLVILK	1617.06	4.10	4 - 18	4.24
☑	SLHAAAVLLLVILK	1460.96	3.71	5 - 18	3.95
☑	EQPSSPAPVNGSK	1297.64	3.29	19 - 31	3.67
☑	WTYFGPDGENSWSK	1673.72	4.25	32 - 45	3.95
☑	WTYFGPDGENSWSKK	1801.82	4.57	32 - 46	4.24
☑	QFLLTNNGHSVK	1357.72	3.44	86 - 97	3.39
☑	LNLPSDMHIQGLQSR	1708.88	4.33	98 - 112	4.24
☑	SEGLAVLAVLIEMGSFNPSYDK	2340.18	5.94	164 - 185	6.21
☑	IFSHLQHVKYK	1399.78	3.55	186 - 196	3.11
☑	YKGQEAFVPGFNIEELLPER	2336.19	5.93	195 - 214	5.65
☑	TAEYYR	802.37	2.04	215 - 220	1.69
☑	TAEYYRYR	1121.54	2.84	215 - 222	2.26
☑	YRGSLTTPPCNPTVLWTVFR	2308.19	5.85	221 - 240	5.65
☑	EMINNFR	923.44	2.34	269 - 275	1.98
☑	EMINNFRQVQK	1406.72	3.57	269 - 279	3.11
☑	EMINNFRQVQKFDER	1953.96	4.96	269 - 283	4.24
☑	QVQKFDER	1049.54	2.66	276 - 283	2.26
☑	RKSIK	631.42	1.60	329 - 333	1.41
☑	KSIKK	603.42	1.53	330 - 334	1.41
☑	SIKKGDNK	889.51	2.26	331 - 338	2.26
☑	KGDNKGVIYK	1121.63	2.84	334 - 343	2.82
☑	GDNKGVIYK	993.54	2.52	335 - 343	2.54
☑	GVIYKPATKMETEAHA	1745.89	4.43	339 - 354	4.52
☑	PATKMETEAHA	1185.56	3.01	344 - 354	3.11
☑	METEAHA	788.32	2.00	348 - 354	1.98

Enhanced signature peptide (ESP) results for CA12 (Note: ESP ranking is not included in this table)

This is a list of *at most* the top 5 peptides predicted to be the highest responding, from each protein, along with up to 5 transitions for multiple charge states

The transitions are based on an LTQ with collision energy at 30

Acc.	m/z	transition	sequence	z	y-ion type
CA12_Isoform1	488.7949	416.25	GVIYKPATK	2	y4
CA12_Isoform1	488.7949	707.41	GVIYKPATK	2	y6
CA12_Isoform1	488.7949	410.75	GVIYKPATK	2	y7++
CA12_Isoform1	488.7949	544.35	GVIYKPATK	2	y5
CA12_Isoform1	488.7949	820.49	GVIYKPATK	2	y7
CA12_Isoform1	326.199	410.75	GVIYKPATK	3	y7++
CA12_Isoform1	326.199	354.21	GVIYKPATK	3	y6++
CA12_Isoform1	326.199	416.25	GVIYKPATK	3	y4
CA12_Isoform1	326.199	272.68	GVIYKPATK	3	y5++
CA12_Isoform1	326.199	460.28	GVIYKPATK	3	y8++
CA12_Isoform1	854.9436	684.84	LNLPSDMHIQGLQSR	2	y12++
CA12_Isoform1	854.9436	1368.67	LNLPSDMHIQGLQSR	2	y12
CA12_Isoform1	854.9436	801.46	LNLPSDMHIQGLQSR	2	y7
CA12_Isoform1	854.9436	535.28	LNLPSDMHIQGLQSR	2	y9++
CA12_Isoform1	854.9436	688.37	LNLPSDMHIQGLQSR	2	y6
CA12_Isoform1	570.2982	684.84	LNLPSDMHIQGLQSR	3	y12++
CA12_Isoform1	570.2982	741.38	LNLPSDMHIQGLQSR	3	y13++
CA12_Isoform1	570.2982	535.28	LNLPSDMHIQGLQSR	3	y9++
CA12_Isoform1	570.2982	798.4	LNLPSDMHIQGLQSR	3	y14++
CA12_Isoform1	570.2982	801.46	LNLPSDMHIQGLQSR	3	y7
CA12_Isoform1	427.9754	390.21	LNLPSDMHIQGLQSR	4	y3
CA12_Isoform1	427.9754	560.32	LNLPSDMHIQGLQSR	4	y5
CA12_Isoform1	427.9754	262.15	LNLPSDMHIQGLQSR	4	y2
CA12_Isoform1	427.9754	684.84	LNLPSDMHIQGLQSR	4	y12++
CA12_Isoform1	427.9754	280.66	LNLPSDMHIQGLQSR	4	y5++
CA12_Isoform1	679.3648	856.43	QFLLTNNGHSVK	2	y8
CA12_Isoform1	679.3648	969.51	QFLLTNNGHSVK	2	y9
CA12_Isoform1	679.3648	1082.6	QFLLTNNGHSVK	2	y10
CA12_Isoform1	679.3648	755.38	QFLLTNNGHSVK	2	y7
CA12_Isoform1	679.3648	541.8	QFLLTNNGHSVK	2	y10++
CA12_Isoform1	453.2456	485.26	QFLLTNNGHSVK	3	y9++
CA12_Isoform1	453.2456	541.8	QFLLTNNGHSVK	3	y10++
CA12_Isoform1	453.2456	428.72	QFLLTNNGHSVK	3	y8++
CA12_Isoform1	453.2456	378.19	QFLLTNNGHSVK	3	y7++
CA12_Isoform1	453.2456	615.33	QFLLTNNGHSVK	3	y11++
CA12_Isoform1	340.186	428.72	QFLLTNNGHSVK	4	y8++
CA12_Isoform1	340.186	378.19	QFLLTNNGHSVK	4	y7++
CA12_Isoform1	340.186	485.26	QFLLTNNGHSVK	4	y9++
CA12_Isoform1	340.186	321.17	QFLLTNNGHSVK	4	y6++
CA12_Isoform1	340.186	264.15	QFLLTNNGHSVK	4	y5++

CA12_Isoform1	730.9818	630.92	SLHAAAVLLLVILK	2	y12++
CA12_Isoform1	730.9818	811.6	SLHAAAVLLLVILK	2	y7
CA12_Isoform1	730.9818	698.52	SLHAAAVLLLVILK	2	y6
CA12_Isoform1	730.9818	585.43	SLHAAAVLLLVILK	2	y5
CA12_Isoform1	730.9818	910.67	SLHAAAVLLLVILK	2	y8
CA12_Isoform1	487.657	585.43	SLHAAAVLLLVILK	3	y5
CA12_Isoform1	487.657	698.52	SLHAAAVLLLVILK	3	y6
CA12_Isoform1	487.657	472.35	SLHAAAVLLLVILK	3	y4
CA12_Isoform1	487.657	373.28	SLHAAAVLLLVILK	3	y3
CA12_Isoform1	487.657	811.6	SLHAAAVLLLVILK	3	y7
CA12_Isoform1	837.3652	1076.46	WTYFGPDGENSWSK	2	y10
CA12_Isoform1	837.3652	1019.44	WTYFGPDGENSWSK	2	y9
CA12_Isoform1	837.3652	1223.53	WTYFGPDGENSWSK	2	y11
CA12_Isoform1	837.3652	807.36	WTYFGPDGENSWSK	2	y7
CA12_Isoform1	837.3652	1386.6	WTYFGPDGENSWSK	2	y12
CA12_Isoform1	558.5792	510.22	WTYFGPDGENSWSK	3	y9++
CA12_Isoform1	558.5792	538.73	WTYFGPDGENSWSK	3	y10++
CA12_Isoform1	558.5792	621.3	WTYFGPDGENSWSK	3	y5
CA12_Isoform1	558.5792	404.18	WTYFGPDGENSWSK	3	y7++
CA12_Isoform1	558.5792	612.27	WTYFGPDGENSWSK	3	y11++