2008 ABRF Proteomics Sample Analysis Report

A. Experimental

Sample (*Adapted from ABRF sample description*) – The study samples are supplied in two vials (labeled "A" and "B") and consist of separate preparations of related human proteins that were expressed in *Escherichia coli* and affinity purified. The cloning vectors that were used produced an N-terminal His6-tagged fusion protein that included a thrombin cleavage site in the His-tag. In both preparations, the sequence GSHM was retained with the protein after thrombin cleavage to remove the Histag.

Vials A and B contain 5 µg and 10 µg of protein, respectively. The samples were prepared from aqueous solutions that also contained small amounts of salts. To the best of our knowledge, there are no appreciable quantities of interfering substances that contain primary amines and/or free thiols. The samples have been successfully dissolved in 25 – 50 mM ammonium bicarbonate with or without 20% acetonitrile; as well as 0.1% formic acid; we anticipate that other solvents can be used.

Protein Sample Solution Preparation – Sample A was dissolved in 20 µl of 0.1% trifluoroacetic acid (TFA) in water containing 10 % acetonitrile (ACN). Sample B was dissolved in 40 µl of same solvent as above.

SDS-PAGE Analysis – 10 µl of Sample-A and 20 µl of Sample-B were dried by speed-vac and resuspend in SDS sample buffer (1X). 2 µl of 1.2 M dithiothreitol (DTT) was added to give final concentration of 10 mM. Sample was boiled for 5 min. After sample was cooled to room temperature, 1 µl of 1 M iodoacetamide was added to final concentration of 50 mM. Sample was kept in dark at room temperature for 40 min. A 4-20% Tris-glycine gel was used to separate the proteins. Colloidal commassie blue was used to stain proteins (3 hours staining and overnight destaining with water).

In-Gel Trypsin Digestion – Protein bands were cut out and destained with acetonitrile / 100mM ammonium bicarbonate (1:1, v/v). Before trypsin digestion, gel pieces were dried by adding 50 µl of acetonitrile. Trypsin, 100 ng/band (Roche), and 50 µl of 100mM ammonium bicarbonate were added and incubated at 37 ºC for overnight. 50 µl of POROS 20 R2 beads (Applied Biosystems) in 5% formic acid/ 0.2%TFA (1:20, v/v) was added to each sample and shaked for about 4 hours and proceed with C18 zip-tip clean-up.

In-Solution Trypsin Digestion – The remaining of samples (9 µl of Sample-A and 19 µl of Sample-B respectively) were further diluted with 50 mM ammonium bicarbonate (pH 8.0) up to 40 µl. Protein was reduced by adding 0.4 µl of 1 M of tris(2-Carboxyethyl) phosphine (TCEP) to final concentration of 10mM and incubated at 37 ºC for 60 min. Reduced protein was alkylated by adding 2 µl of 1 M of iodoacetamide to final concentration of 50mM and incubated at room temperature for 60 min in the dark. Following alkylation, trypsin digestion was carried out by adding 1 µl and 2 µl of trypsin

solution (0.1µg/µl, Promega) into Sample-A and Sample-B, respectively, and incubated at 37°C overnight in the dark. Digested sample solution was acidified by adding 1 µl of 5% of TFA. Tryptic peptide samples were desalted using C18 zip-tip.

MALDI-TOF-MS Analysis of Protein – Solution (1 µl) of Sample-A and sample-B were mixed with 9 µl of matrix solution containing α-Cyano-4-hydroxycinnamic acid (4HCCA) in formic acid/isopropanol/water (FWI 3:1:2) separately. Protein sample spots were prepared using the 'thin-layer' MALDI sample preparation method (ref). Myoglobin was used as an external calibrant. The samples and the calibrant were analyzed by a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Voyager DE-STR, Applied Biosystems) operating in linear delayed extraction mode. One thousand individual scans were averaged prior to acquisition into a single spectrum. The spectra were smoothed and further analyzed using the software M-over-Z.

MALDI-TOF-MS Analysis of Tryptic Peptides – Each sample was resuspended with 7 µl of 0.1% formic acid containing 2% acetonitrile. 0.5 µl of each sample was mixed with 2 µl of 4HCCA in 0.1% TFA of water/acetonitrile (1:1, v/v) and spotted on a MALDI target plate. The samples were analyzed by a MALDI-TOF mass spectrometer (Voyager DE-STR, Applied Biosystems) operating in linear and reflector mode.

For in-solution tryptic digestion sample, each sample was resuspended with 10 µl of 2% acetonitrile, 0.1% formic acid. 1 µl of each sample was mixed with 4 µl of 4HCCA in in 0.1% TFA of water/acetonitrile (1:1, v/v) and spotted on a MALDI target plate. The samples were analyzed as mentioned above.

LC-MS/MS Analysis of Tryptic Peptide – All digested peptide mixtures were separated by an online reversed-phase (RP) nano liquid chromatography (nanoLC) and analyzed by electrospray tandem mass spectrometry (ESI-MS/MS) using a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific). The samples were injected with a Shimadzu SIL-20AC auto-sampler onto a nano-RP column (75µm x 15 cm, 3 µm, 300 Á, Micro-Tech Scientific). A 30 min gradient from 2% to 75% acetonitrile was used for in-gel digested samples and a 60 min gradient from 2% to 75% acetonitrile was used for in-solution digested samples. Data-dependent acquisition was performed on the LTQ-Orbitrap mass spectrometer in the positive ion mode. Survey MS scans were acquired in the orbitrap with the resolution of 60,000. Up to 5 most intense ions per cycle were fragmented and analyzed in the linear ion trap. Target ions already selected for MS/MS were dynamically excluded for 180 s. HPLC solvent A is 0.1% formic acid in water containing 2% of ACN and solvent B is 0.1% formic acid in ACN containing 2% of water.

Database Search – For analysis of MALDI-TOF-MS data, PROWL was used. Peptide masses were searched against NCBI nr database (Homo sapiens) with mass error tolerance of 75 ppm. For analysis of MS/MS data, Sequest was used on a Socerer 2 computer. MS/MS data was searched against Swiss Homo sapiens database with precursor mass error tolerance of 10 ppm.

All MS/MS samples were analyzed using Sequest (ThermoFinnigan, version v.27, rev.11). Sequest was set up to search the swissprot database (UniProtKB release 12.0 of 24-Jul-2007, 141396 entries) assuming the digestion enzyme trypsin. Sequest was search with a fragment ion mass tolerance of 1.00 Da and a parent ion tolerance of 20 ppm. Iodoacetamide derivative of cysteine was specified in Sequest as a fixed modification. Deamidation of asparagine and glutamine, oxidation of histidine, methionine and tryptophan and phosphorylation of serine, threonine and tyrosine were specified in Sequest as variable modifications.

Criteria for protein identification – Scafffold (version Scaffold-01_07_00, Proteome Software, Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability as specified by the Peptide Prophet algorithm (Keller, A. et al, Anal.Chem. 2002, 74(20):5383-92). Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhski, AL, Anal.Chem, 2003, 75(17):4646-58). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony

B. Results

MALDI-TOF-MS Analysis of Protein Samples – The protein samples were first analyzed by matrix-assisted laser desorption time-of-flight mass spectrometry. In MALDI-TOF-MS spectrum of *Sample-A*, a characteristic protein spectrum was observed (Figure 1). Multiple peaks were observed corresponding to different charge states of a single protein. Based on the observed m/z's, the *Sample-A* protein has averaged molecular weight of 33139 Da. In MALDI-TOF-MS spectrum of Sample-B, a protein mixture spectrum was observed. Based on peak distribution analysis, three proteins can be identified with molecular weights of 33120, 24554, and 12524 Da, respectively. The MALDI-TOF-MS analyses of the intact protein samples were summarized in Table 1.

SDS-PAGE Analysis of Protein Samples – To separate the proteins, SDS-PAGE was employed. The protein samples were separated by Tris-glycine gel (Figure 3). As observed in MALDI-TOF-MS spectra, *Sample-A* showed one band and *Sample-B* showed three major bands with two minor smear bands just below band 1 and band 2. Interesting thing is that when comparing with molecular weight marker (SeeBlue Plus2 Pre-Stained Standard, Invitrogen, reference Appendix 1), the apparent molecular weights of proteins were much higher than the molecular weights detected in mass spectrometric analysis (reference to the left-side molecular weight marker). When comparing to the molecular weights of the standard proteins, the SDS-PAGE measured molecular weights were much close to that measured by MALDI-TOF-MS (reference to the right-side molecular weight marker).

Protein Identification – To identify proteins in *Sample-A* and *Sample-B*, in-gel digestion was performed on the protein bands separated by SDS-PAGE. The in-gel digests were first analyzed by MALDI-TOF-MS (Figure 4). Database searching of all of in-gel digests resulted same protein, soluble form of receptor for advanced glycation endproducts (gi|28971760, Homo sapiens). As indicated in Figure 4, the band A01 from *Sample-A* and band B01 (and B01') from *Sample-B* resulted very similar spectra. However, less peaks were detected in the spectra of band B02 (and B02') and B03 of *Sample-B*, which indicated C-terminal truncation of the protein (reference the dotted line and sequence labels). The C-terminal truncation can be clearly seen in peptide sequence alignment diagrams (Figure 5). Detailed peptide mass fingerprint analyses of in-gel digests were summarized in Table 2. The protein identification was further confirmed by LC-MS/MS experiment of the same in-gel digested protein band samples (Table 3 and Figure 6). Again the same protein was identified from all of LC-MS/MS analyses. Although, different accession number (gi|2497317) was assigned to the identified protein than that of MALDI-TOF-MS analysis (gi|28971760), these two sequences share the same amino acid sequence from 1-328.

In-Solution Digestion – To further examine whether there is any other protein presented in the sample, in-solution trypsin digestions of the *Sample-A* and *Sample-B* were performed and analyzed using MALDI-TOF-MS (Figure 7) and LC-MS/MS. Database searching resulted same protein identification as that of in-gel digests (Table 4 and 5). The sequence alignments were summarized in Figure 8 and 9. No other protein was identified from both samples.

C. Conclusion

Sample A contains one protein, soluble form of receptor for advanced glycation endproducts (gi|28971760, Homo sapiens).

Sample B contains the same protein as Sample A with two additional C-terminal truncated products.

Table 1. Molecular mass calculation of based on MALDI-TOF-MS analysis

Sampe	Protein	m/z	z	mass	Mol.Mass
A	A	33155	1	33154	33139
		16574	2	33146	
		11047	3	33138	
		8284.3	4	33133	
		6627.0	5	33130	
		5523.5	6	33135	
B	B1	33134	1	33133	33120
		16566	2	33130	
		11041	3	33120	
		8279.4	4	33114	
		6623.3	5	33112	
		5519.5	6	33111	
	B2	24559	1	24558	24554
		12279	2	24556	
		8184.0	3	24549	
		6139.1	4	24552	
	B3	12524	1	12523	12524
		6263.3	2	12525	
		4175.4	3	12523	

Table 2. Peptide mass figure print analysis of in-gel digests

Sample	Protein Band	Accession #	Protein ID	Score	Sequence Coverage (%)	Peptides							
						Measured Mass(M)	*Computed Mass*	*Error (ppm)*	*Residues Start*	*Residues To*	*Missed Cut*	*Peptide sequence*	*Modification*
A	A01	gi|28971760	soluble form of receptor for advanced glycation endproducts		60	867.538	867.542	-5	30	37	0	IGEPLVLK	
						1180.67	1180.671	-1	44	52	1	KPPQRLEWK	
						1174.605	1174.609	-3	53	62	1	LNTGRTEAWK	
						1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
						2241.214	2241.215	0	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
						1451.703	1451.66	29	99	110	2	CQAMNRNGKETK	(1)+O@M
						2511.288	2511.321	-13	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
						3097.444	3097.516	-23	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
						2298.138	2298.178	-17	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
						2142.059	2142.077	-8	180	198	0	HPETGLFTLQSELMVTPAR	(1)+O@M
						1933.904	1933.91	-3	199	216	0	GGDPRPTFSCSFSPGLPR	
						4332.018	4332.146	-30	274	314	0	DGVPLPLPPSPVLILPEIGPQDQGTYSCVATHSSHGPQESR	
B	B01	gi|28971760	soluble form of receptor for advanced glycation endproducts		61	867.531	867.542	-13	30	37	0	IGEPLVLK	
						1180.667	1180.671	-3	44	52	1	KPPQRLEWK	
						1174.601	1174.609	-7	53	62	1	LNTGRTEAWK	
						1524.767	1524.768	0	63	77	0	VLSPQGGGPWDSVAR	
						2241.214	2241.215	0	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
						1451.7	1451.66	28	99	110	2	CQAMNRNGKETK	(1)+O@M
						2511.269	2511.321	-21	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
						3097.457	3097.516	-19	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
						2298.148	2298.178	-13	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
						1933.907	1933.91	-2	199	216	0	GGDPRPTFSCSFSPGLPR	
						4332.051	4332.146	-22	274	314	0	DGVPLPLPPSPVLILPEIGPQDQGTYSCVATHSSHGPQESR	
						1297.592	1297.571	16	337	347	1	VREAEDSPQHM	
	B01'	gi|28971760	soluble form of receptor for advanced glycation endproducts		57	867.495	867.542	-54	30	37	0	IGEPLVLK	
						1174.583	1174.609	-22	53	62	1	LNTGRTEAWK	
						1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
						2241.186	2241.215	-13	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
						1451.673	1451.66	9	99	110	2	CQAMNRNGKETK	(1)+O@M
						2511.279	2511.321	-17	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
						3097.368	3097.516	-48	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
						2298.147	2298.178	-14	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
						1933.884	1933.91	-13	199	216	0	GGDPRPTFSCSFSPGLPR	
						4332.078	4332.146	-16	274	314	0	DGVPLPLPPSPVLILPEIGPQDQGTYSCVATHSSHGPQESR	
	B02	gi|28971760	soluble form of receptor for advanced glycation endproducts		45	867.492	867.542	-58	30	37	0	IGEPLVLK	
						1174.58	1174.609	-25	53	62	1	LNTGRTEAWK	
						1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
						241.171	2241.215	-20	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
						1451.687	1451.66	18	99	110	2	CQAMNRNGKETK	(1)+O@M
						2511.371	2511.321	20	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
						3097.559	3097.516	14	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
						2298.201	2298.178	10	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
						1933.916	1933.91	3	199	216	0	GGDPRPTFSCSFSPGLPR	
	B02'	gi|28971760	soluble form of receptor for advanced glycation endproducts		45	867.513	867.542	-34	30	37	0	IGEPLVLK	
						1174.625	1174.609	14	53	62	1	LNTGRTEAWK	
						1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
						2241.241	2241.215	12	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
						1451.695	1451.66	24	99	110	2	CQAMNRNGKETK	(1)+O@M
						2511.312	2511.321	-4	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
						3097.444	3097.516	-23	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
						2298.148	2298.178	-13	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
						2142.077	2142.077	0	180	198	0	HPETGLFTLQSELMVTPAR	(1)+O@M
						1933.909	1933.91	0	199	216	0	GGDPRPTFSCSFSPGLPR	
	B03	gi|28971760	soluble form of receptor for advanced glycation endproducts		18	867.515	867.542	-31	30	37	0	IGEPLVLK	
						1180.658	1180.671	-11	44	52	1	KPPQRLEWK	
						1730.874	1730.91	-21	49	62	2	LEWKLNTGRTEAWK	
						1174.613	1174.609	3	53	62	1	LNTGRTEAWK	
						1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
						2241.214	2241.215	0	78	98	0	VLPNGSLFLPAVGIQDEGIFR	

Table 3. Summary of Protein database searching results of LC-MS/MS analysis of in-gel digests

Sample	Protein Band	Protein name	Protein accession numbers	Protein molecular weight (Da)	Protein identification probability	Number of unique peptides	Percentage sequence coverage	Peptide sequence	Previous amino acid	Next amino acid	Best Peptide identification probability	Best SEQUEST XCorr score	Best SEQUEST DCn score	Calculated Peptide Mass (AMU)	Peptide start index	Peptide stop index
A	A01	Advanced glycosylation end product-specific	gi|2497317| sp|Q15109| RAGE_HU	42783.8	100.00%	5	20.30%	VLSPQGGGPWDSVAR	K	V	95.00%	3.2	0.401	1525.7764	63	77
								VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	K	G	95.00%	3.44	0.341	3098.5259	141	169
								RHPETGLFTLQSELMVTPAR	R	G	95.00%	2.69	0.411	2299.187	179	198
								GGDPRPTFSCSFSPGLPR	R	H	95.00%	3.8	0.429	1934.9187	199	216
								PTFSCSFSPGLPR	R	H	95.00%	3.36	0.607	1452.6947	204	216
B	B01	Spectra corrupted														
	B01'	Advanced glycosylation end product-specific	gi|2497317| sp|Q15109| RAGE_HU	42783.8	100.00%	3	8.17%	VLSPQGGGPWDSVAR	K	V	95.00%	3.44	0.291	1525.7764	63	77
								GGDPRPTFSCSFSPGLPR	R	H	95.00%	3.29	0.273	1934.9187	199	216
								PTFSCSFSPGLPR	R	H	95.00%	3.54	0.613	1452.6947	204	216
	B02	Advanced glycosylation end product-specific	gi|2497317| sp|Q15109| RAGE_HU	42783.8	100.00%	7	28.20%	IGEPLVLK	R	C	95.00%	1.82	0.285	868.551	30	37
								VLSPQGGGPWDSVAR	K	V	95.00%	3.24	0.365	1525.7764	63	77
								VYQIPGKPEIVDSASELTAGVPNK	R	V	95.00%	2.93	0.215	2512.3301	117	140
								VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	K	G	95.00%	4.05	0.301	3098.5259	141	169
								RHPETGLFTLQSELMVTPAR	R	G	95.00%	4.36	0.431	2299.187	179	198
								GGDPRPTFSCSFSPGLPR	R	H	95.00%	2.31	0.289	1934.9187	199	216
								PTFSCSFSPGLPR	R	H	95.00%	3.57	0.608	1452.6947	204	216
	B02'	Advanced glycosylation end product-specific	gi|2497317| sp|Q15109| RAGE_HU	42783.8	100.00%	3	14.10%	VLSPQGGGPWDSVAR	K	V	95.00%	3.65	0.298	1525.7764	63	77
								VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	K	G	95.00%	4.84	0.471	3114.5207	141	169
								PTFSCSFSPGLPR	R	H	95.00%	3.29	0.544	1452.6947	204	216
	B03	Advanced glycosylation end product-specific	gi|2497317| sp|Q15109| RAGE_HU	42783.8	100.00%	3	10.90%	IGEPLVLK	R	C	95.00%	2.64	0.33	868.551	30	37
								VLSPQGGGPWDSVAR	K	V	95.00%	3.22	0.372	1525.7764	63	77
								VLPNGSLFLPAVGIQDEGIFR	R	C	95.00%	3.35	0.458	2243.208	78	98

Table 4. Peptide mass figure print analysis of in-solution digests

Sample	Accession #	Protein ID	Score	Sequence Coverage (%)	Peptides							
					Measured Mass(M)	*Computed Mass*	*Error (ppm)*	*Residues Start*	*Residues To*	*Missed Cut*	*Peptide sequence*	*Modification*
A	gi|28971760	soluble form of receptor for advanced glycation endproducts		57	867.495	867.542	-55	30	37	0	IGEPLVLK	
					1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
					2241.245	2241.215	14	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
					1451.676	1451.66	11	99	110	2	CQAMNRNGKETK	(1)+O@M
					2511.347	2511.321	10	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
					3097.561	3097.516	14	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
					2282.239	2282.183	24	179	198	1	RHPETGLFTLQSELMVTPAR	
					2298.276	2298.178	42	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
					2126.013	2126.082	-33	180	198	0	HPETGLFTLQSELMVTPAR	
					1933.917	1933.91	4	199	216	0	GGDPRPTFSCSFSPGLPR	
					1121.637	1121.666	-26	219	228	1	ALRTAPIQPR	
					4332.137	4332.146	-2	274	314	0	DGVPLPLPPSPVLILPEIGPQDQGTYSCVATHSSHGPQESR	
B	gi|28971760	soluble form of receptor for advanced glycation endproducts		61	867.561	867.542	21	30	37	0	IGEPLVLK	
					1730.871	1730.91	-22	49	62	2	LEWKLNTGRTEAWK	
					1524.767	1524.768	-1	63	77	0	VLSPQGGGPWDSVAR	
					2241.179	2241.215	-16	78	98	0	VLPNGSLFLPAVGIQDEGIFR	
					1451.682	1451.66	15	99	110	2	CQAMNRNGKETK	(1)+O@M
					2511.226	2511.321	-38	117	140	0	VYQIPGKPEIVDSASELTAGVPNK	
					3097.371	3097.516	-47	141	169	0	VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	
					2282.139	2282.183	-19	179	198	1	RHPETGLFTLQSELMVTPAR	
					2298.177	2298.178	-1	179	198	1	RHPETGLFTLQSELMVTPAR	(1)+O@M
					2126.045	2126.082	-17	180	198	0	HPETGLFTLQSELMVTPAR	
					1933.852	1933.91	-30	199	216	0	GGDPRPTFSCSFSPGLPR	
					4331.881	4332.146	-61	274	314	0	DGVPLPLPPSPVLILPEIGPQDQGTYSCVATHSSHGPQESR	
					1297.593	1297.571	17	337	347	1	VREAEDSPQHM	

Table 5. Summary of Protein database searching results of LC-MS/MS analysis of in-solution digests

Sample	Protein name	Protein accession numbers	Protein molecular weight (Da)	Protein identification probability	Number of unique peptides	Percentage sequence coverage	Peptide sequence	Previous amino acid	Next amino acid	Best Peptide identification probability	Best SEQUEST XCorr score	Best SEQUEST DCn score	Calculated Peptide Mass (AMU)	Peptide start index	Peptide stop index
A	Advanced glycosylation end product-specific	gi|2497317|sp|Q15109|RAGE_HUMA	42783.8	100.00%	10	30.20%	IGEPLVLK	R	C	95.00%	1.93	0.372	868.551	30	37
							IGEPLVLKCK	R	G	95.00%	2.41	0.274	1213.6982	30	39
							TEAWKVLSPQGGGPWDSVAR	R	V	95.00%	5.39	0.506	2141.0781	58	77
							VLSPQGGGPWDSVAR	K	V	95.00%	3.37	0.588	1525.7764	63	77
							VLPNGSLFLPAVGIQDEGIFR	R	C	95.00%	2.55	0.285	2242.2239	78	98
							VYQIPGKPEIVDSASELTAGVPNK	R	V	95.00%	3.01	0.307	2512.3301	117	140
							VGTCVSEGSYPAGTLSWHLDGK	K	P	95.00%	3.56	0.362	2321.0874	141	162
							VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	K	G	95.00%	4.06	0.412	3098.5259	141	169
							GGDPRPTFSCSFSPGLPR	R	H	95.00%	4.22	0.455	1934.9187	199	216
							PTFSCSFSPGLPR	R	H	95.00%	3.88	0.641	1452.6947	204	216
B	Advanced glycosylation end product-specific	gi|2497317|sp|Q15109|RAGE_HUMA	42783.8	100.00%	8	28.50%	IGEPLVLK	R	C	95.00%	2.44	0.248	868.551	30	37
							VLSPQGGGPWDSVAR	K	V	95.00%	3.46	0.201	1525.7764	63	77
							VLPNGSLFLPAVGIQDEGIFR	R	C	95.00%	4.92	0.648	2242.2239	78	98
							VYQIPGKPEIVDSASELTAGVPNK	R	V	95.00%	2.83	0.285	2512.3301	117	140
							VGTCVSEGSYPAGTLSWHLDGK	K	P	95.00%	4.59	0.472	2321.0874	141	162
							VGTCVSEGSYPAGTLSWHLDGKPLVPNEK	K	G	95.00%	4.29	0.384	3098.5259	141	169
							GGDPRPTFSCSFSPGLPR	R	H	95.00%	4.59	0.395	1934.9187	199	216
							PTFSCSFSPGLPR	R	H	95.00%	3.7	0.535	1452.6947	204	216

MALDI-TOF-MS Spectrum of Sample-A

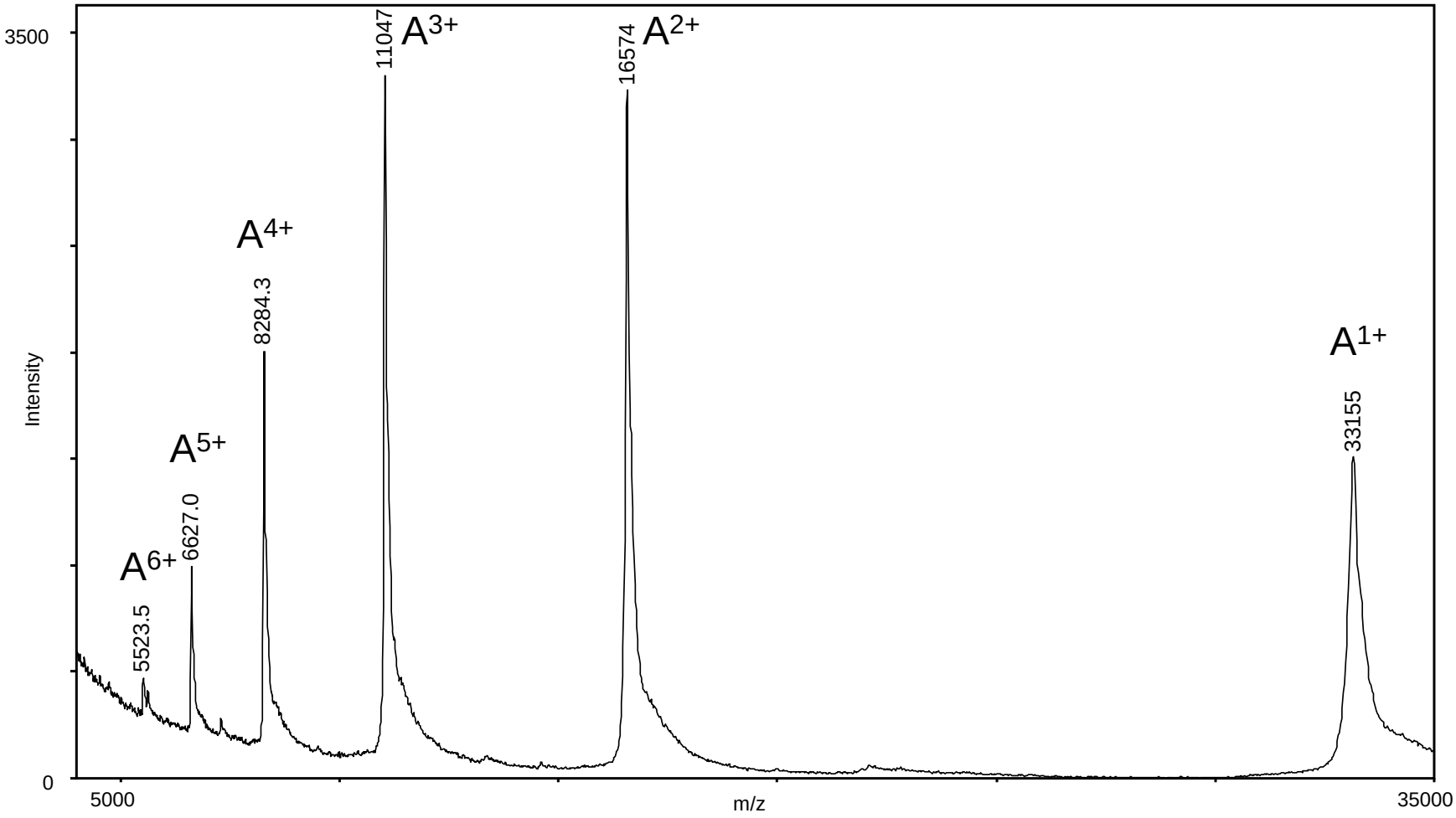


Figure 1

MALDI-TOF-MS Spectrum of Sample-B

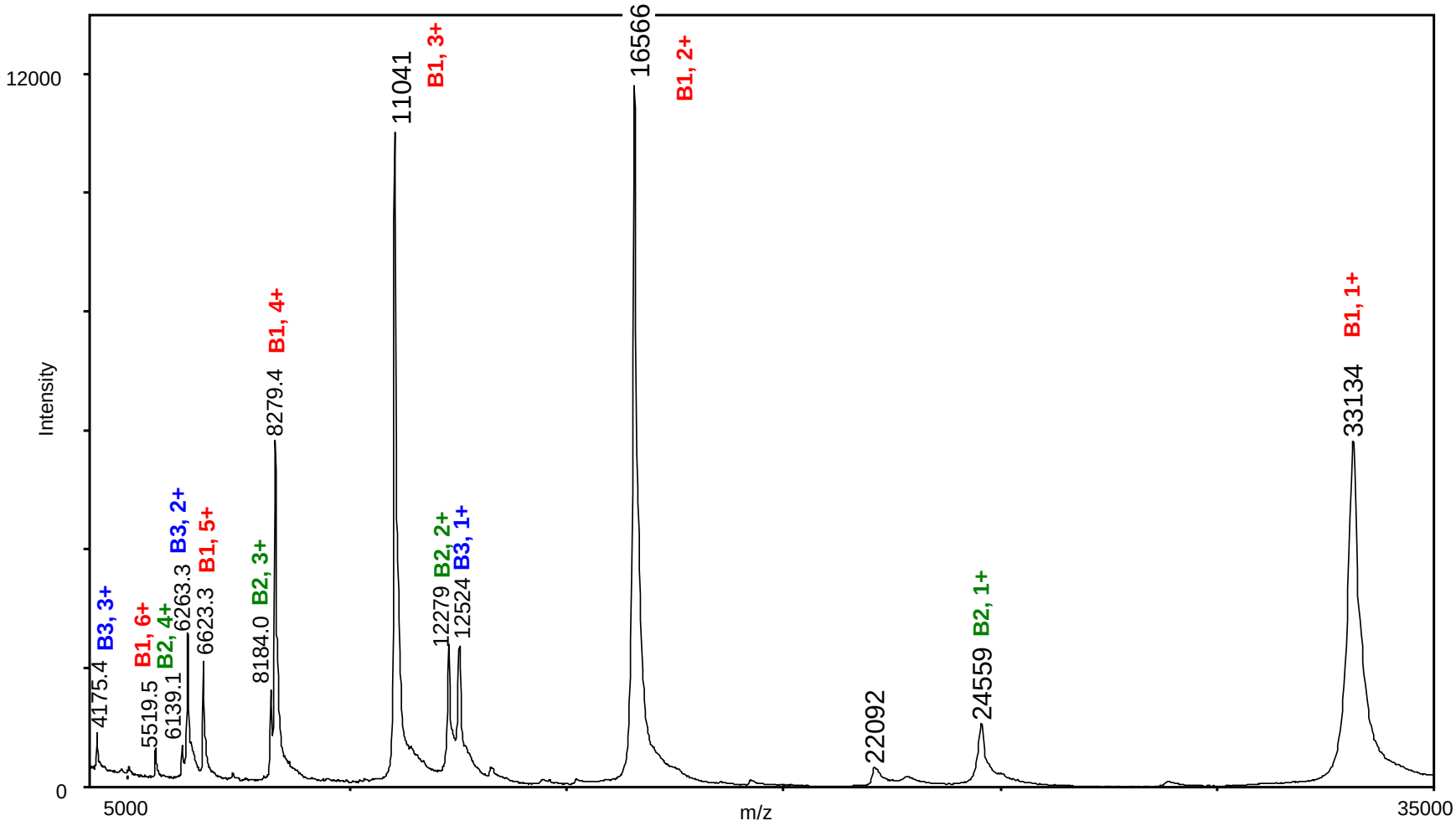


Figure 2

SDS-PAGE Analysis of Samples

Tris-Glycine Gel, Reduction/alkylation before running the gel

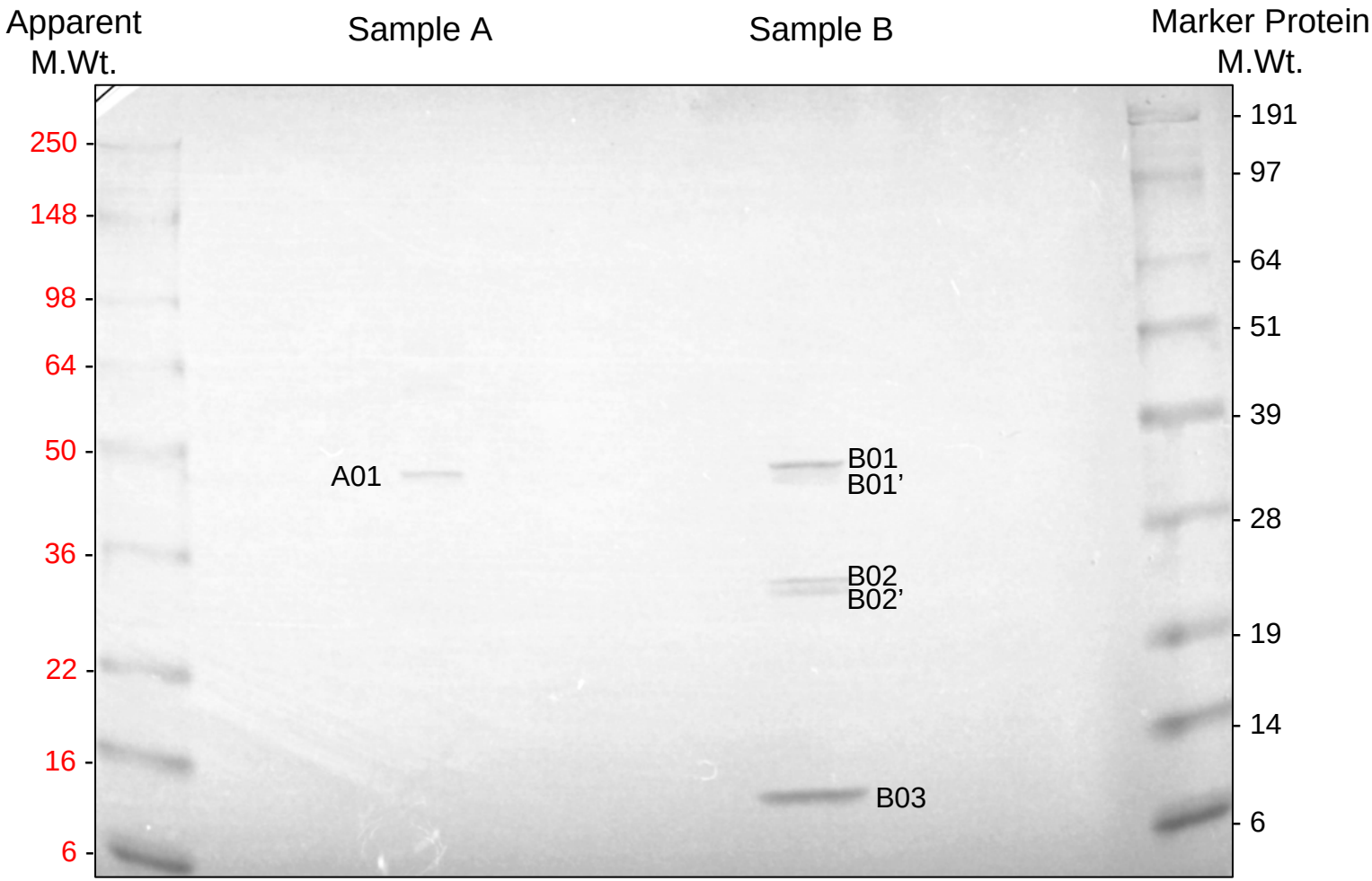


Figure 3

MALDI-TOF-MS of In-Gel Digests

Analysis against GSHM-gi|28971760

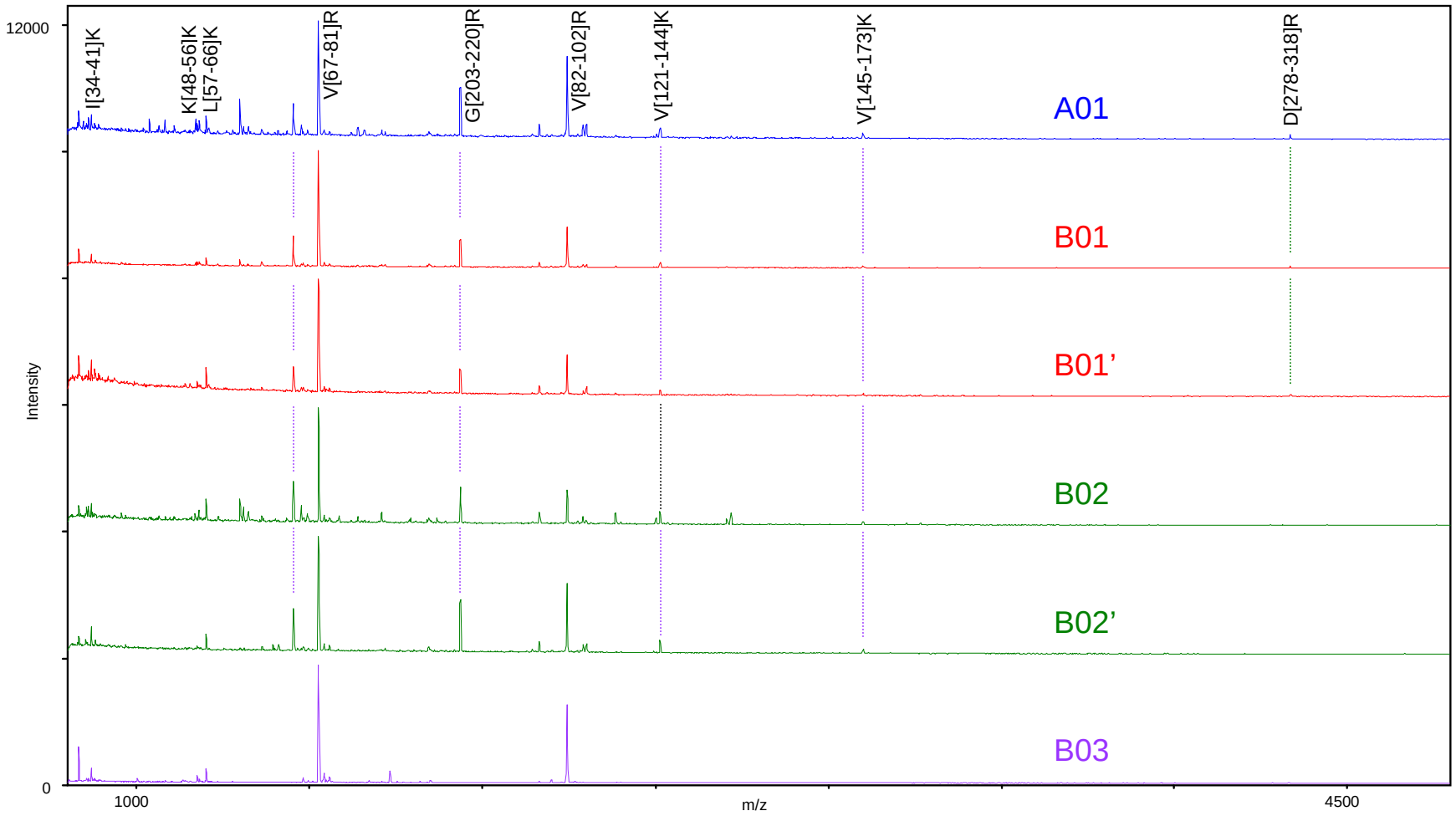
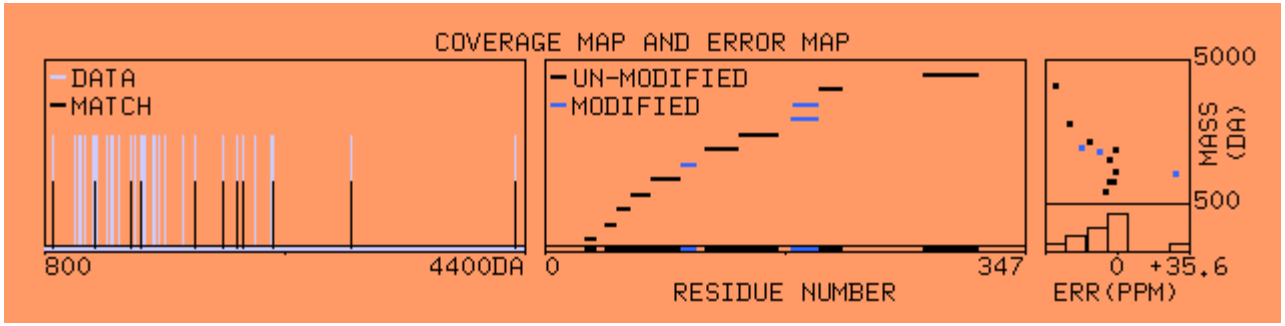


Figure 4

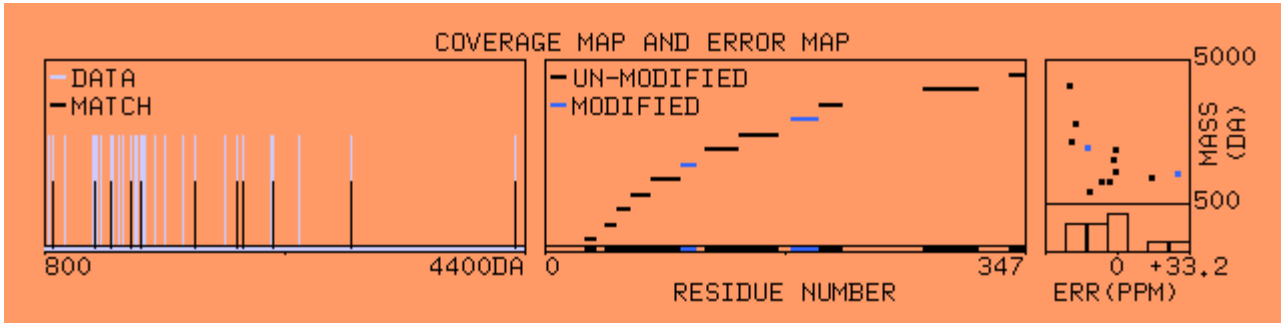
gi|28971760, soluble form of receptor for advanced glycation endproducts [*H.sapiens*]

Tryptic Peptide Matching Analysis

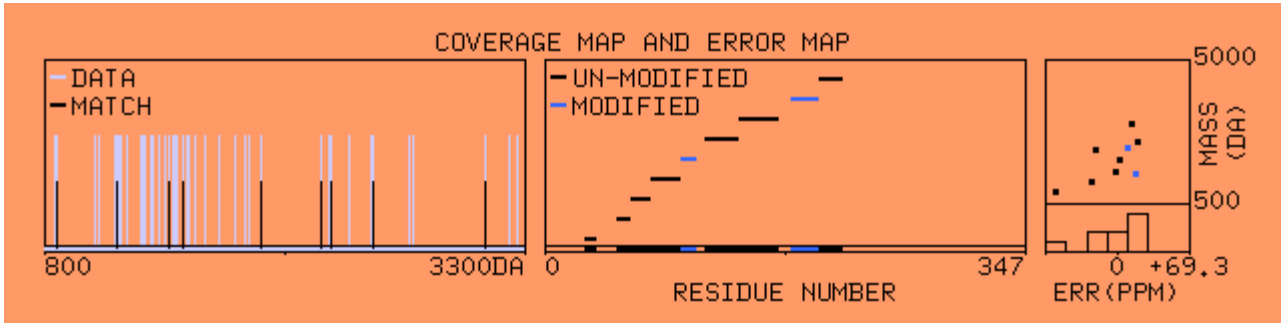
A01



B01



B02



B03

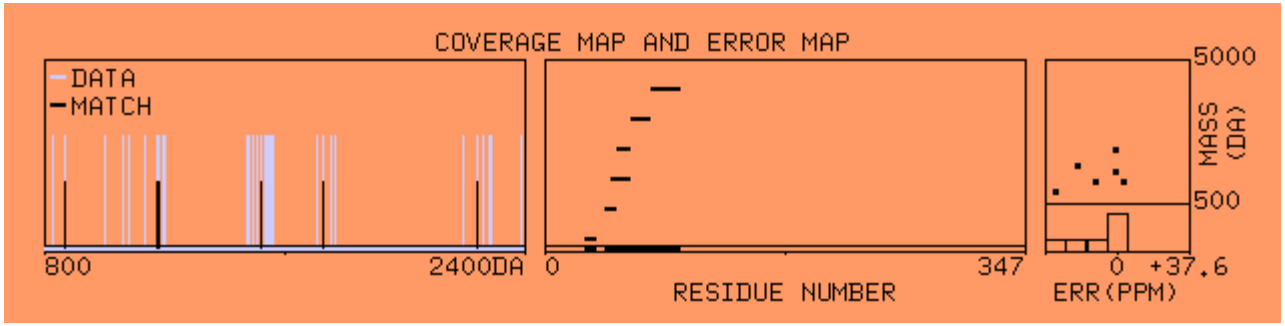
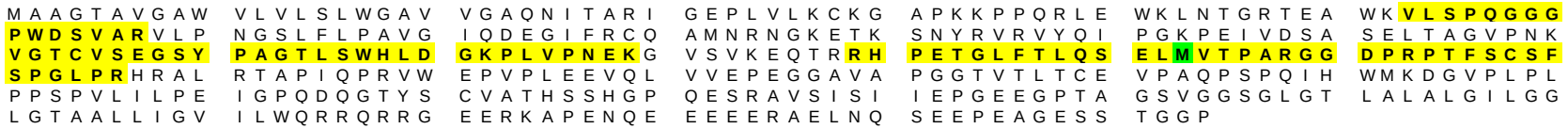


Figure 5

LC-MS/MS Analyses of In-Gel Digests

Sequence Coverage Analysis against gi|2497317

A01



B01 Due to Microsoft Window automatic update during overnight run, MS data collection was interrupted that resulted data corruption and no protein identification.

B01'

B02

B02'

B03

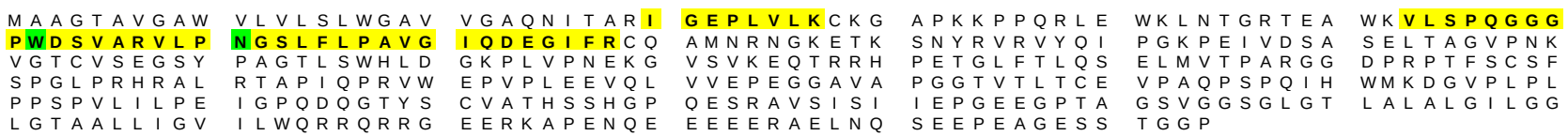


Figure 6

MALDI-TOF-MS of In-Solution Digests

Analysis against GSHM-gi|28971760

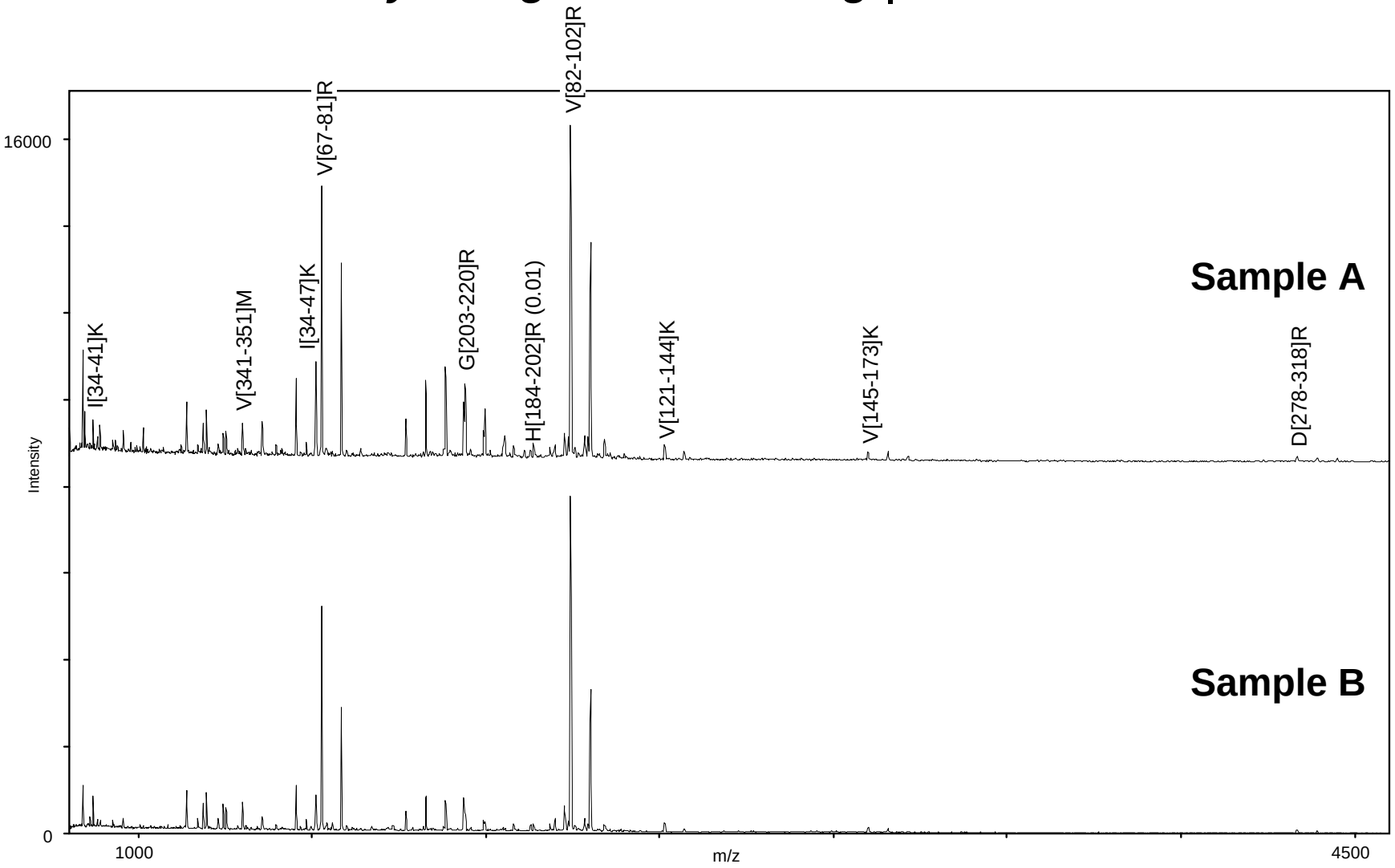
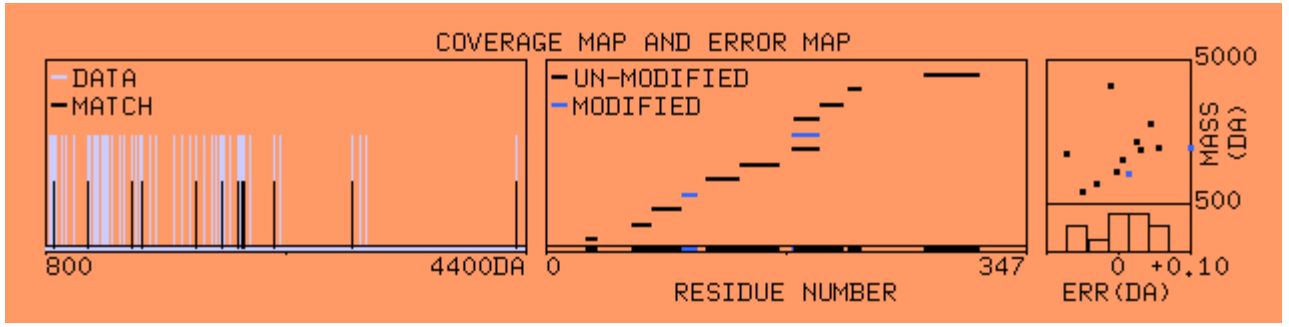


Figure 7

Tryptic Peptide Matching Analysis

Analysis against gi|28971760

Sample A



Sample B

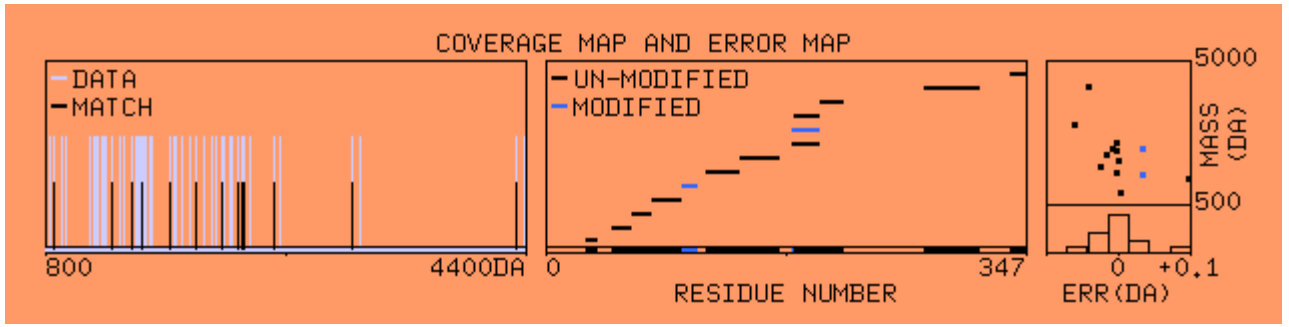


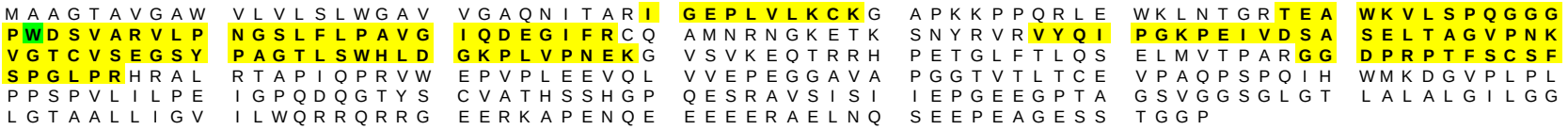
Figure 8

LC-MS/MS Analyses of In-Solution Digests

Sequence Coverage Analysis against gi|2497317

Sample A

gi|2497317|sp|Q15109|RAGE_HUMAN (100%), 42801.9 Da
gi|2497317|sp|Q15109|RAGE_HUMAN
10 unique peptides, 16 unique spectra, 27 total spectra, 122/404 amino acids (30% coverage)



Sample B

gi|2497317|sp|Q15109|RAGE_HUMAN (100%), 42801.9 Da
gi|2497317|sp|Q15109|RAGE_HUMAN
8 unique peptides, 13 unique spectra, 29 total spectra, 115/404 amino acids (28% coverage)

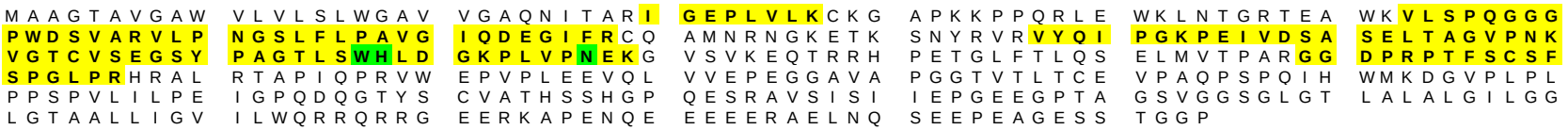


Figure 9

SeeBlue® Plus2 Pre-Stained Standard

Invitrogen, Catalog no. LC5925

Example

The apparent molecular weights of the protein bands in SeeBlue® Plus2 Pre-Stained Standard in several buffer systems is shown below. The protein bands have different mobilities in various SDS-PAGE buffer systems. For more information on this phenomenon, contact technical service (see previous page) or visit our Web site at www.invitrogen.com.

NuPAGE® Novex Bis-Tris 4-12% Gel

Protein	Approximate Molecular Weights (kDa)				
	Tris-Glycine	Tricine	NuPAGE® MES	NuPAGE® MOPS	NuPAGE® Tris-Acetate
Myosin	250	210	188	191	210
Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

IM-1008F 072602

Appendix 1