

ABRF-PRG08 Proteomics Research group (PRG) Qualitative Proteomics Study

Method applied: 1D-Gel electrophoresis, in-gel digest, peptide mass fingerprint

Methods:

1D-Gel electrophoresis:

Sample dissolved in 25 mM NH_4HCO_3

Sample (1 μg in 6 μl) boiled 5 min after addition of 2 μl sample buffer (4 fold concentrated)

1 μg from Sample A and B applied on a 12.5% acrylamide SDS-PAGE

Staining: Flamingo

Lane detection and excision:

Spot-Cutter (BioRAD) under UV illumination with Quantity One software

In-gel digestion:

MicroStarlet (Hamilton Robotics) with in house protocol:

10 min RT 150 μl 50% acetonitrile

2 min RT 150 μl 100% acetonitrile

Reduction with DTT (10 mM) 30 min at 56°C

10 min 56°C 200 μl 100% acetonitrile

Alkylation RT 15 μl IAA (10 mM)

5 min RT 150 μl NH_4HCO_3 (50 mM)

5 min RT 150 μl 100% acetonitrile

17 μl 8°C Trypsin (10 ng/ml)

50 μl 8°C NH_4HCO_3

Removal of 55 μl supernatant

Digestion 120 min 45°C

10 μl 0.1% TFA (0.1% octylglycoside)

Storage of collected supernatant at 4°C

MALDI-TOF-MS:

Ultraflex I ToF/ToF (Bruker Daltonics)

HCCA with citric acid

Flex Analysis 3.0 and BioTools 3.1, MASCOT 2.2; MSDB_20060831.fasta

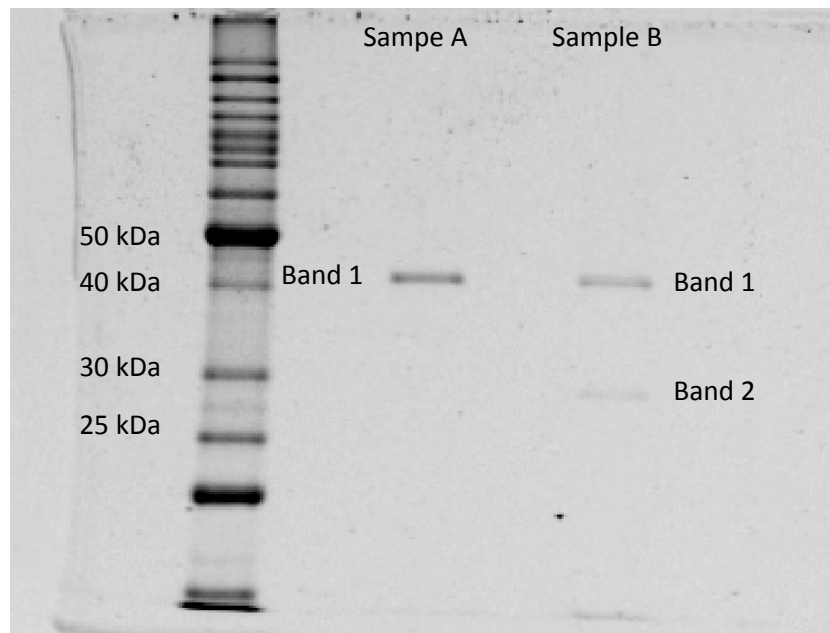
Search criteria after statistical calibration and removal of background peaks:

first pass:

species human, Trypsin/P, 100 ppm deviation, fixed modification carbamidomethylation, optional modification methionine oxidation

second pass:

using RAGE sequence (Q15109 including GSHM tag) an in-silico digest of the protein was performed including deamination as further optional modification

Results:**Gel image**

Sample A: one band at approx. 42 kDa

Sample B: one band at approx. 42 kDa

one band at approx. 28 kDa

Identifications:

First pass:

Sampe	Protein	Mowse score	Sequence coverage
A, Band 1	RAGE AAA03574	145	46.5 %
B, Band 1	RAGE AAA03574	127	43.3 %
C, Band 2	RAGE AAA03574	109	34.2 %

Second pass:

Sampe	Protein	Mowse score	Sequence coverage
A, Band 1	RAGE		46.2 %
B, Band 1	RAGE		43 %
C, Band 2	RAGE		35.1 %

Conclusion:

Both samples contain RAGE, but sample B additionally a truncated form of RAGE.

Appendix:

BioTools data from the analyses

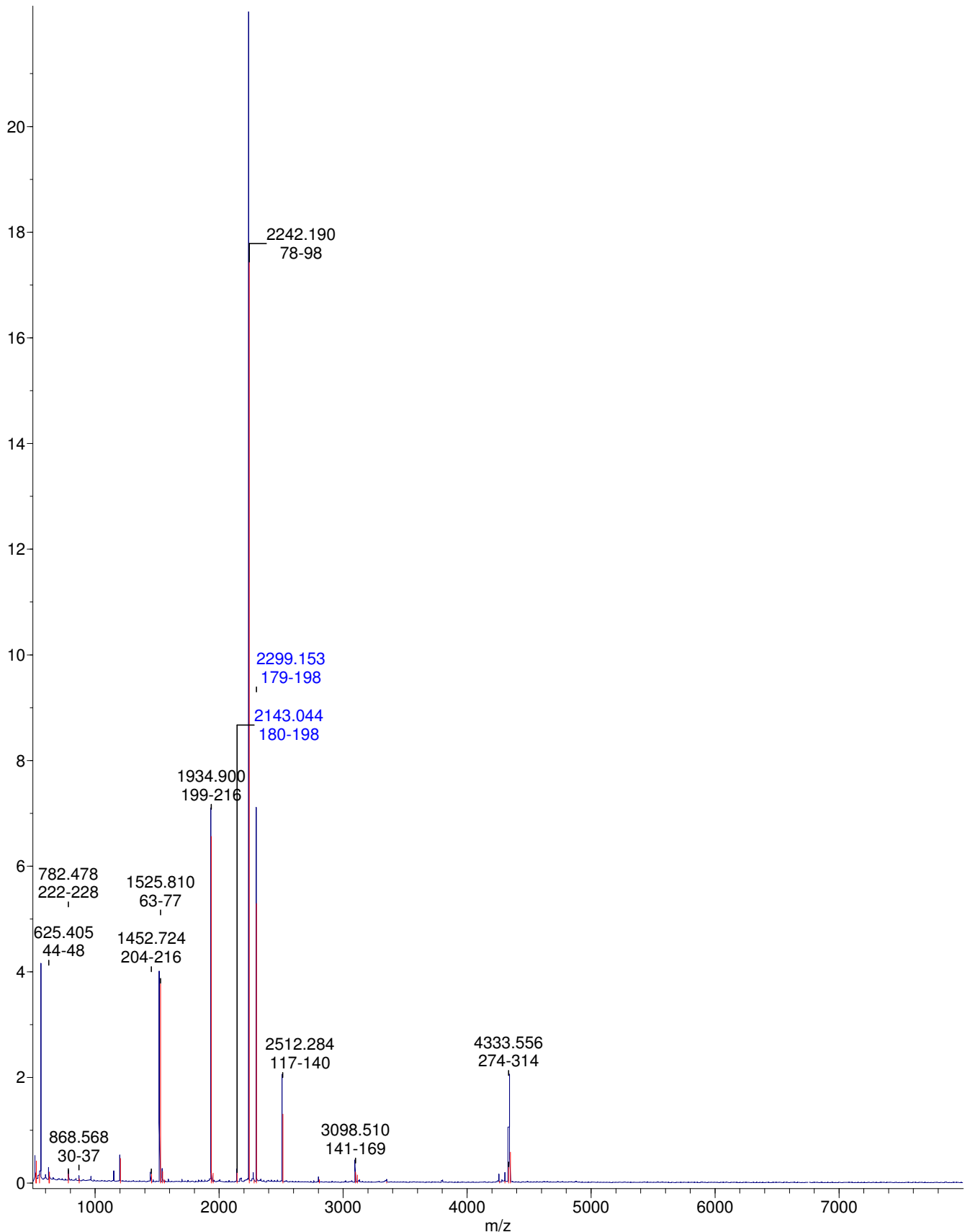
Spectrum Analysis Report

Date: 11/30/2007 Time: 13:52

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample A\0_I22\1\1SRef\data\11r

Sequence Name:	HUMRAGE NID: - Homo sapiens AAA03574	Parentmass:	
Formula:		MH+ (mono):	1.008
Mass Error:		Threshold (a.i.):	0.000
MH+ (avg):	1.008	Number of Peaks:	27
Tolerance (Da):	0.500	Assigned Peaks:	
Above Threshold:			
Not assigned Peaks:			

Abs. Int. * 1000



Spectrum Analysis Report

Date: 11/30/2007 Time: 13:52

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample A\0_I22\1\1SRef\data\11r

Sequence data:

HUMRAGE NID: - Homo sapiens AAA03574

Intensity Coverage: 92.5 % (34381 cnts)
 Sequence Coverage MS/MS: 0.0%

Sequence Coverage MS: 46.5%
 pI (isoelectric point): 6.0

10	20	30	40	50	60	70	80	90
GAAGTAVGAW	VLVLSLWGAV	VGAQNTTARI	GEPLVLKCKG	APKKPPQRL	WKLNTGRTEA	WKVLSPPQGG	PWDSVARVLP	NGSLFLPAVG
100	110	120	130	140	150	160	170	180
IQDEGIFRCR	AMNRNGKETK	SNYRVRVYQI	PGKPEIVDSA	SELTAGVPNK	VGTCVSEGSY	PAGTLSWHL	GKPLVPNEK	VSVKEQTRRH
190	200	210	220	230	240	250	260	270
PETGLFTLOS	ELMVTPARGG	DPRPTFSCSF	SPGLPRHRAL	RTAPIQPRVW	EPVPLEEVQL	VVEPEGGA	PGGTVTLTCE	VPAQPSPQIH
280	290	300	310	320	330	340	350	360
WMKDGVPLPL	PPSPVLILPE	IGPQDQGTYS	CVATHSSHGP	QESRAVSISI	IEPGEEGPTA	GSVGGSGLGT	LALALGILGG	LGTAAALLIGV
370	380	390	400	410				
ILWQRQR	EERKAPENQE	EEEEAEELNQ	SEEPAGESS	TGGP				

Display Parameter:

Sequence Name:	HUMRAGE NID: - Homo sapiens AAA03574		
MH+ (mono):	1.008	MH+ (avg):	1.008
Threshold (a.i.):	0.000	Tolerance (Da):	0.500
Number of Peaks:	27		

Peaklist:

Peak	Mass	Intensity	Peak	Mass	Intensity
1	520.292	182.994	2	524.263	407.416
3	550.145	160.543	4	625.405	112.809
5	628.649	203.133	6	782.478	168.890
7	868.568	82.049	8	1201.614	454.224
9	1452.724	163.980	10	1525.810	3642.735
11	1541.789	210.955	12	1557.740	59.869
13	1934.900	6331.727	14	1948.917	182.194
15	2143.044	193.611	16	2242.190	16822.769
17	2280.143	56.418	18	2299.153	5106.807
19	2512.284	1261.794	20	2807.289	67.342
21	3098.510	204.298	22	3112.528	148.062
23	3348.639	23.333	24	4260.517	35.897
25	4302.666	38.269	26	4333.556	289.984
27	4347.596	567.821			

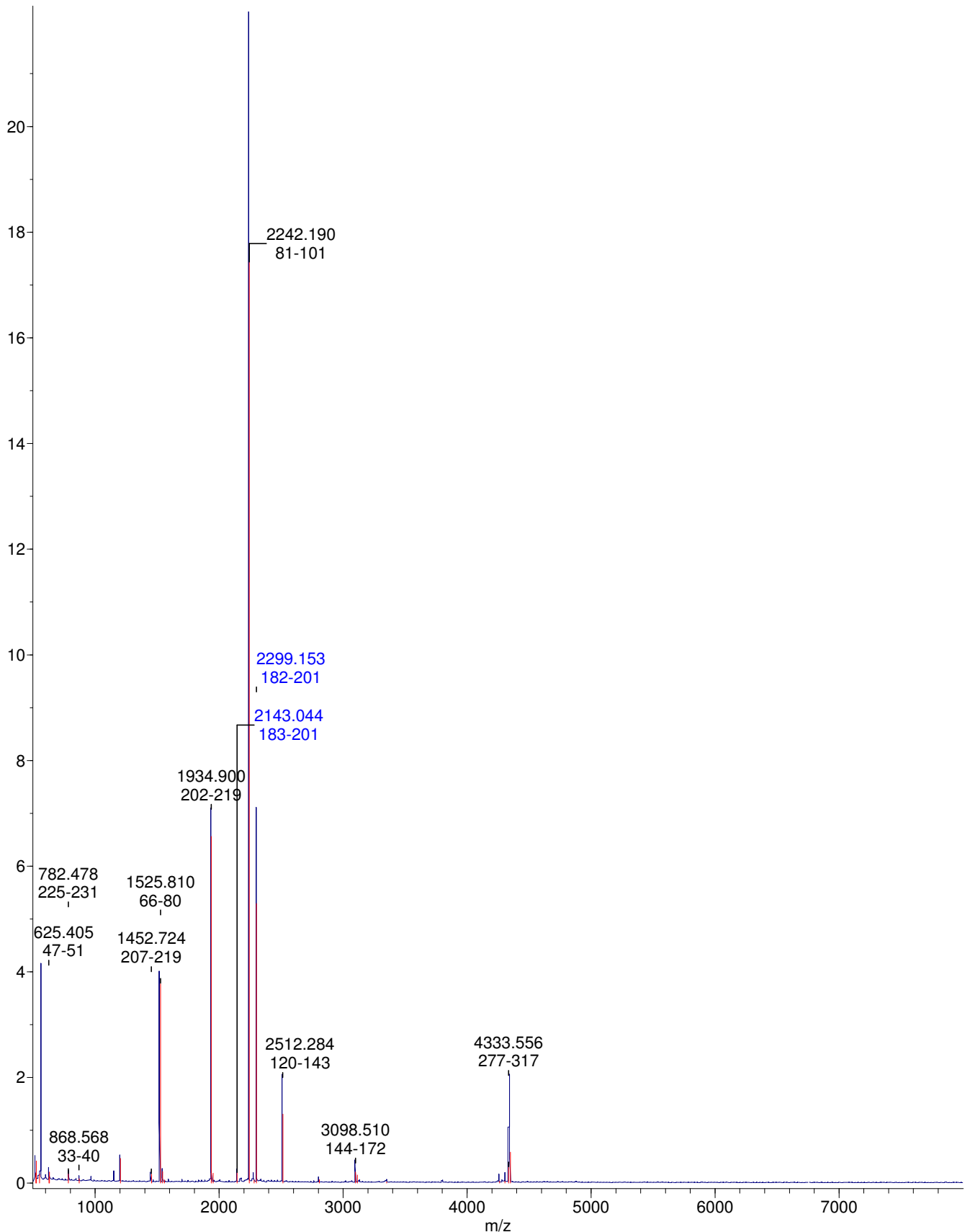
Spectrum Analysis Report

Date: 11/30/2007 Time: 13:53

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample A\0_I22\1\1SRef\data\11r

Sequence Name:	RAGE Human + Tag:Amino Acids		
Formula:		Parentmass:	
Mass Error:		MH+ (mono):	1.008
MH+ (avg):	1.008	Threshold (a.i.):	0.000
Tolerance (Da):	0.500	Number of Peaks:	27
Above Threshold:		Assigned Peaks:	
Not assigned Peaks:			

Abs. Int. * 1000



Spectrum Analysis Report

Date: 11/30/2007 Time: 13:53

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample A\0_I22\1\1SRef\data\11r

Sequence data:

RAGE Human + Tag:Amino Acids

Intensity Coverage: 92.5 % (34381 cnts)
 Sequence Coverage MS/MS: 0.0%

Sequence Coverage MS: 46.2%
 pI (isoelectric point): 5.9

10	20	30	40	50	60	70	80	90
GSHMAAGTAV	GAWVLVLSLW	GAVVGAQ NIT	ARIGEPVLVK	CKGAPK KPPQ	RLEWKLNTGR	TEAWK VLSPO	GGGPWDSVAR	VLP NGSLFLP
100	110	120	130	140	150	160	170	180
AVGIQDEGIF	RCQAMNRRNGK	ETKSNYRVRV	YQIPGKPEIV	DSASELTAGV	PNKVGT CVSE	GSYPAGTLSW	HLDGKPLVPM	EKGVS VKEQT
190	200	210	220	230	240	250	260	270
RRHPETGLFT	LQSELMVTPA	RGGDPRPTFS	CSFSPGLPRH	RALRTAPIQP	RVWEPVPLEE	VQLVVEPEGG	AVAPGGTVTL	TCEVPAQSP
280	290	300	310	320	330	340	350	360
QIHWMKDGVP	LPLPPSPVLI	LPEIGPDQOG	TYSCVATHSS	HGPQESRAVS	ISIIEPGEEG	PTAGSVGGSG	LGTLALALGI	LGGLGTAALL
370	380	390	400	410				
IGVILWQRRQ	RRGEERKAPE	NQEEEEERAE	L NS EEPEAG	ESSTGGP				

Display Parameter:

Sequence Name:	RAGE Human + Tag:Amino Acids		
MH+ (mono):	1.008	MH+ (avg):	1.008
Threshold (a.i.):	0.000	Tolerance (Da):	0.500
Number of Peaks:	27		

Peaklist:

Peak	Mass	Intensity	Peak	Mass	Intensity
1	520.292	182.994	2	524.263	407.416
3	550.145	160.543	4	625.405	112.809
5	628.649	203.133	6	782.478	168.890
7	868.568	82.049	8	1201.614	454.224
9	1452.724	163.980	10	1525.810	3642.735
11	1541.789	210.955	12	1557.740	59.869
13	1934.900	6331.727	14	1948.917	182.194
15	2143.044	193.611	16	2242.190	16822.769
17	2280.143	56.418	18	2299.153	5106.807
19	2512.284	1261.794	20	2807.289	67.342
21	3098.510	204.298	22	3112.528	148.062
23	3348.639	23.333	24	4260.517	35.897
25	4302.666	38.269	26	4333.556	289.984
27	4347.596	567.821			

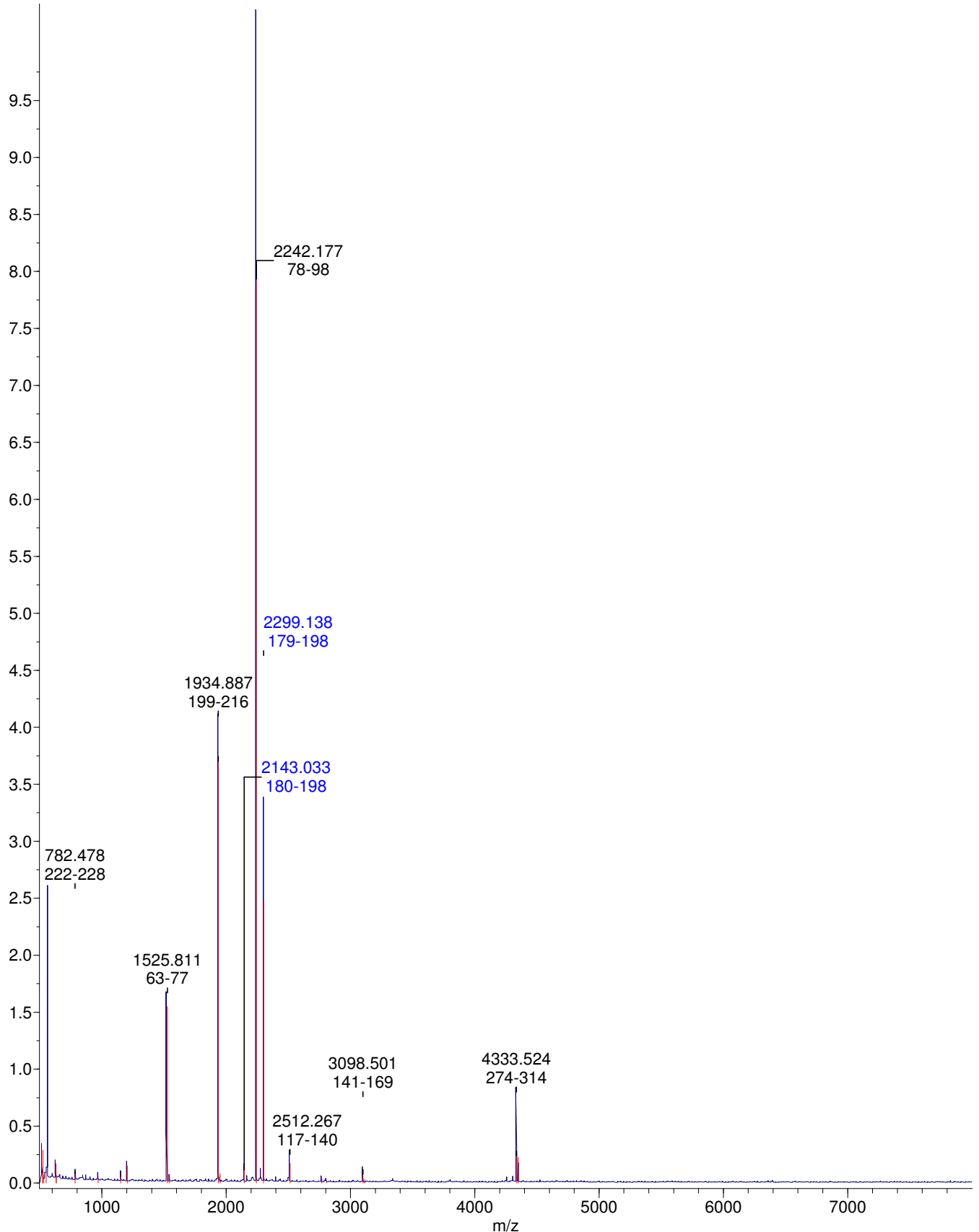
Spectrum Analysis Report

Date: 11/30/2007 Time: 13:54

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_J22\1\1SRef\data\1\1r

Sequence Name:	HUMRAGE NID: - Homo sapiens	AAA03574	Parentmass:	
Formula:			MH+ (mono):	1.008
Mass Error:			Threshold (a.i.):	0.000
MH+ (avg):	1.008		Number of Peaks:	24
Tolerance (Da):	0.200		Assigned Peaks:	
Above Threshold:				
Not assigned Peaks:				

Abs. Int. * 1000



Spectrum Analysis Report

Date: 11/30/2007 Time: 13:54

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_J22\1\1SRef\data\1\1r

Sequence data:

HUMRAGE NID: - Homo sapiens AAA03574

Intensity Coverage: 91.6 % (15764 cnts)
 Sequence Coverage MS/MS: 0.0%

Sequence Coverage MS: 43.3%
 pI (isoelectric point): 6.0

10	20	30	40	50	60	70	80	90	100
GAAGTAVGAW	VLVLSLWGAV	VGAQNTTARI	GEPLVLKCKG	APKKPPQRLE	WKLNTGRTEA	WKVLSPOGGG	PWDSVARVLP	NGSLFLPAVG	IQDEGIFRCR
110	120	130	140	150	160	170	180	190	200
AMNRNGKETK	SNYRVRVYQI	PGKPEIVDSA	SELTAGVPNK	VGTCVSEGSY	PAGTLSWHLD	GKPLVPNEKG	VSVKEQTRRH	PETGLFTLQS	ELMVTARGG
210	220	230	240	250	260	270	280	290	300
DPRPTFSCSF	SPGLPRHRAL	RTAPIQPRVM	EPVPLEEVQL	VVEPEGGAVA	PGGTVTLTCE	VPAQPSPQIH	WMKDGVPPLPL	PPSPVLILPE	IGPDQQTYS
310	320	330	340	350	360	370	380	390	400
CVATHSSHGP	QESRAVSISI	IEPGEEGPTA	GSVGGSGLGT	LALALGILGG	LGTAALLIGV	ILWQRRQRRG	EERKAPENQE	EEEEAEELNQ	SEEPAGESS
410									
TGGP									

Display Parameter:

Sequence Name:	HUMRAGE NID: - Homo sapiens AAA03574		
MH+ (mono):	1.008	MH+ (avg):	1.008
Threshold (a.i.):	0.000	Tolerance (Da):	0.200
Number of Peaks:	24		

Peaklist:

Peak	Mass	Intensity	Peak	Mass	Intensity
1	522.244	60.212	2	524.250	278.440
3	526.285	68.345	4	550.146	93.111
5	628.641	165.095	6	631.366	64.033
7	782.478	71.522	8	968.478	57.603
9	1150.600	56.202	10	1201.612	146.563
11	1525.811	1495.883	12	1541.787	63.664
13	1934.887	3568.449	14	1948.909	80.642
15	2143.033	111.630	16	2242.177	7654.550
17	2299.138	2396.408	18	2512.267	168.687
19	2767.470	36.071	20	2807.297	20.587
21	3098.501	69.620	22	3112.514	29.475
23	4333.524	226.989	24	4347.561	218.508

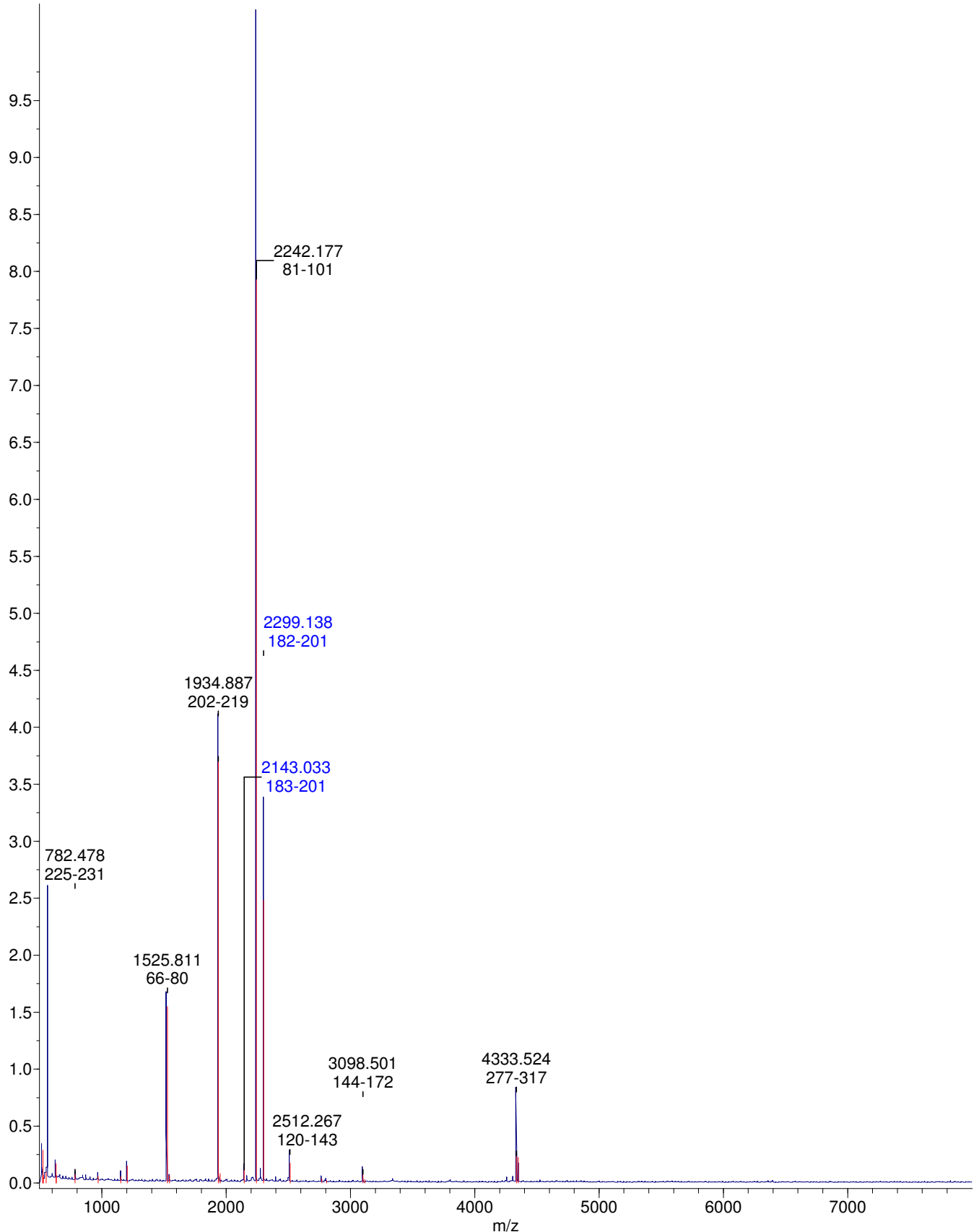
Spectrum Analysis Report

Date: 11/30/2007 Time: 13:55

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_J22\1\1SRef\data\1\1r

Sequence Name:	RAGE Human + Tag:Amino Acids		
Formula:		Parentmass:	
Mass Error:		MH+ (mono):	1.008
MH+ (avg):	1.008	Threshold (a.i.):	0.000
Tolerance (Da):	0.200	Number of Peaks:	24
Above Threshold:		Assigned Peaks:	
Not assigned Peaks:			

Abs. Int. * 1000



Spectrum Analysis Report

Date: 11/30/2007 Time: 13:55

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_J22\1\1SRefpdata\1\1r

Sequence data:

RAGE Human + Tag:Amino Acids

Intensity Coverage: 91.6 % (15764 cnts)
 Sequence Coverage MS/MS: 0.0%

Sequence Coverage MS: 43.0%
 pI (isoelectric point): 5.9

10	20	30	40	50	60	70	80	90	100
GSHMAAGTAV	GAWVLVLSLW	GAVVGAQNT	ARIGEPLVLK	CKGAPKKPPQ	RLEWKLNTGR	TEAWKVLSPQ	GGGPWDSVAR	VLPNGSLFLP	AVGIQDEGIF
110	120	130	140	150	160	170	180	190	200
RCQAMNRNGK	ETKSNYRVRV	YQIPGKPEIV	DSASELTAGV	PNKVGTCVSE	GSYPAGTLSW	HLDGKPLVPN	EKGVSVKEQT	RRHPETGLFT	LOSELMVTPA
210	220	230	240	250	260	270	280	290	300
RGGDPRTFS	CSFSPGLPRH	RALRTAPIQP	RVWEPVPLEE	VQLWVEPEGG	AVAPGGTVTL	TCEVPAQPSP	QIHWMKDGVP	LPLPPSPVLI	LPEIGPDQG
310	320	330	340	350	360	370	380	390	400
TYSCVATHSS	HGPQESRAVS	ISIIIEPGEEG	PTAGSVGGSG	LGTALALGI	LGLLGTAAAL	IGVILWQRRQ	RRGEERKAPE	NQEEEEERAE	LNQSEEPQAG
410									
ESSTGGP									

Display Parameter:

Sequence Name:	RAGE Human + Tag:Amino Acids		
MH+ (mono):	1.008	MH+ (avg):	1.008
Threshold (a.i.):	0.000	Tolerance (Da):	0.200
Number of Peaks:	24		

Peaklist:

Peak	Mass	Intensity	Peak	Mass	Intensity
1	522.244	60.212	2	524.250	278.440
3	526.285	68.345	4	550.146	93.111
5	628.641	165.095	6	631.366	64.033
7	782.478	71.522	8	968.478	57.603
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11	1525.811	1495.883	12	1541.787	63.664
13	1934.887	3568.449	14	1948.909	80.642
15	2143.033	111.630	16	2242.177	7654.550
17	2299.138	2396.408	18	2512.267	168.687
19	2767.470	36.071	20	2807.297	20.587
21	3098.501	69.620	22	3112.514	29.475
23	4333.524	226.989	24	4347.561	218.508

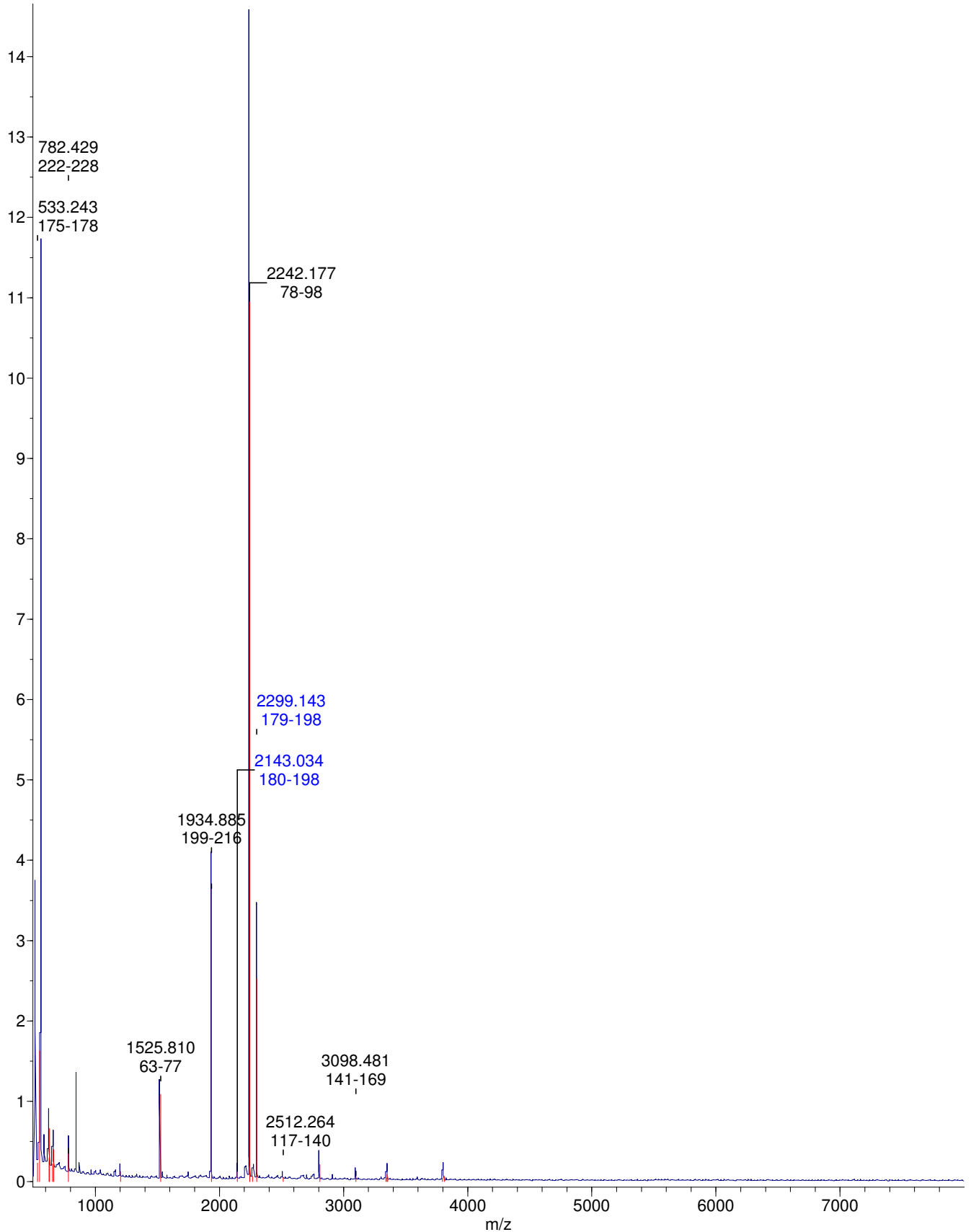
Spectrum Analysis Report

Date: 11/30/2007 Time: 13:56

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_K22\1\1SRef\data\1\1r

Sequence Name:	HUMRAGE NID: - Homo sapiens	AAA03574	Parentmass:	
Formula:			MH+ (mono):	1.008
Mass Error:			Threshold (a.i.):	0.000
MH+ (avg):	1.008		Number of Peaks:	22
Tolerance (Da):	0.200		Assigned Peaks:	
Above Threshold:				
Not assigned Peaks:				

Abs. Int. * 1000



Spectrum Analysis Report

Date: 11/30/2007 Time: 13:56

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_K22\1\1SRef\data\1\1r

Sequence data:

HUMRAGE NID: - Homo sapiens AAA03574

Intensity Coverage: 82.9 % (18367 cnts)
 Sequence Coverage MS/MS: 0.0%

Sequence Coverage MS: 34.2%
 pI (isoelectric point): 6.0

10	20	30	40	50	60	70	80	90	100
GAAGTAVGAW	VLVLSLWGAW	VGAQNTTARI	GEPLVLKCKG	APKKPPQRLE	WKLNTGRTEA	WKVLSPOGGG	PWDSVARVLP	NGSLFLPAVG	IQDEGIFRCR
110	120	130	140	150	160	170	180	190	200
AMNRNGKETK	SNYRVRVYQI	PGKPEIVDSA	SELTAGVPNK	VGTCVSEGSY	PAGTLSWHLD	GKPLVPNEKG	VSVKEQTRRH	PETGLFTLQS	ELMVTARGG
210	220	230	240	250	260	270	280	290	300
DPRPTFSCSF	SPGLPRHRAL	RTAPIQPRVM	EPVPLEEVQL	VVEPEGGAVA	PGGTVTLTCE	VPAQPSPQIH	WMKDGVPPLPL	PPSPVLILPE	IGPQDQGTYS
310	320	330	340	350	360	370	380	390	400
CVATHSSHGP	QESRAVSISI	IEPGEEGPTA	GSVGGSGLGT	LALALGILGG	LGTAALLIGV	ILWQRRQRRG	EERKAPENQE	EEEEAEELNQ	SEEPAGESS
410									
TGGP									

Display Parameter:

Sequence Name:	HUMRAGE NID: - Homo sapiens AAA03574		
MH+ (mono):	1.008	MH+ (avg):	1.008
Threshold (a.i.):	0.000	Tolerance (Da):	0.200
Number of Peaks:	22		

Peaklist:

Peak	Mass	Intensity	Peak	Mass	Intensity
1	533.243	225.148	2	550.075	1569.005
3	625.314	286.594	4	628.564	638.216
5	653.327	215.934	6	663.365	386.065
7	782.429	334.072	8	1201.580	123.036
9	1525.810	1048.227	10	1934.885	3512.132
11	2143.034	122.602	12	2242.177	10570.618
13	2264.161	63.871	14	2299.143	2440.532
15	2512.264	49.047	16	2807.275	206.005
17	3098.481	64.166	18	3338.737	41.748
19	3348.607	87.732	20	3353.726	57.119
21	3794.855	46.121	22	3808.860	64.326

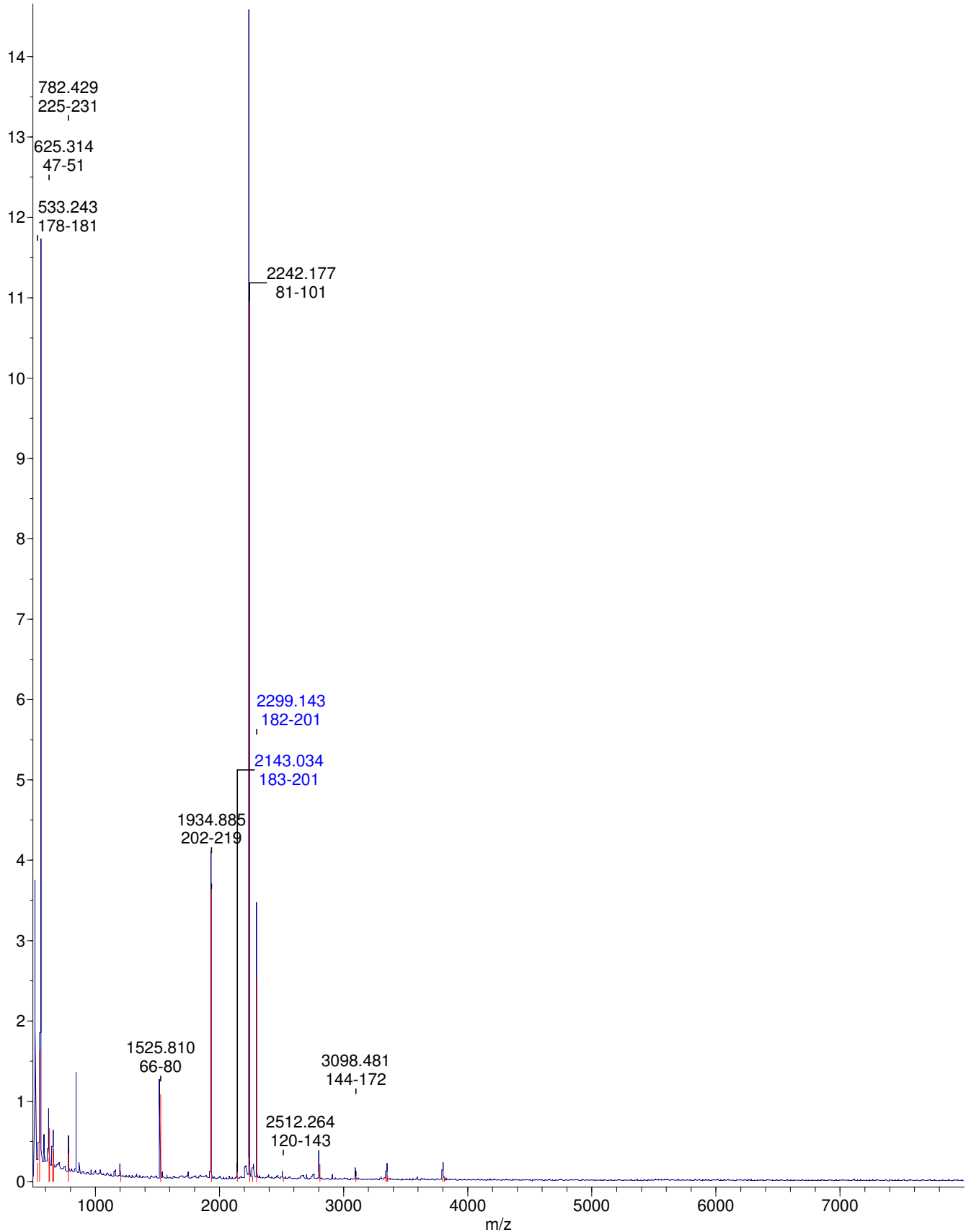
Spectrum Analysis Report

Date: 11/30/2007 Time: 13:56

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_K22\1\1SRef\data\1\1r

Sequence Name:	RAGE Human + Tag:Amino Acids		
Formula:		Parentmass:	
Mass Error:		MH+ (mono):	1.008
MH+ (avg):	1.008	Threshold (a.i.):	0.000
Tolerance (Da):	0.200	Number of Peaks:	22
Above Threshold:		Assigned Peaks:	
Not assigned Peaks:			

Abs. Int. * 1000



Spectrum Analysis Report

Date: 11/30/2007 Time: 13:56

FileName: D:\Data\my_sample\Lochnit\ABRF\Sample B\0_K22\1\1SRef\data\1\1r

Sequence data:

RAGE Human + Tag:Amino Acids

Intensity Coverage: 84.2 % (18653 cnts)
 Sequence Coverage MS/MS: 0.0%

Sequence Coverage MS: 35.1%
 pI (isoelectric point): 5.9

10	20	30	40	50	60	70	80	90	100
GSHMAAGTAV	GAWVLVLSLW	GAVVGAQ NIT	ARIGEPLVLK	CKGAPK KPPQ	RLEWKLNTGR	TEAWK VLS PQ	GGGP WDS VAR	VLP NG SLFLP	AVGIQDEGIF
110	120	130	140	150	160	170	180	190	200
RCQAMN R NGK	ETKS N YRVRV	YQIPG K PEIV	DSASE L TAGV	PNK V GTCVSE	GSYP A GTL S W	HLDG K PLV P N	E K GV S V K E Q T	RR H PETGLFT	LO S ELM V T P A
210	220	230	240	250	260	270	280	290	300
RGG D PR P T F S	CS F SP L PR H	RAL R T A PI Q P	RV W EPV L EE	VQL V VE P EGG	AV A PGG T V T L	TCE V PA Q P S P	QI H W M KD G V P	LPL P SP V L I	LPE I GP Q D Q G
310	320	330	340	350	360	370	380	390	400
TY S CVATH S S	HGP Q ESRA V S	IS I IE P GE E G	PT A GS V GG S G	LG T L A L A L G I	LG L G T A A L L	IG V IL M Q R R Q	RR G EER K A P E	NQ E E E E E R A E	L N Q S E E P E A G
410									
ES S T G G P									

Display Parameter:

Sequence Name:	RAGE Human + Tag:Amino Acids		
MH+ (mono):	1.008	MH+ (avg):	1.008
Threshold (a.i.):	0.000	Tolerance (Da):	0.200
Number of Peaks:	22		

Peaklist:

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9	1525.810	1048.227	10	1934.885	3512.132
11	2143.034	122.602	12	2242.177	10570.618
13	2264.161	63.871	14	2299.143	2440.532
15	2512.264	49.047	16	2807.275	206.005
17	3098.481	64.166	18	3338.737	41.748
19	3348.607	87.732	20	3353.726	57.119
21	3794.855	46.121	22	3808.860	64.326