

# A Centrifugation Assisted Micro-reactor Enables Facile Integration of Trypsin Digestion, HILIC Enrichment and On-column Deglycosylation for Rapid and Sensitive Glycoproteome Analysis

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## SUPPORTING INFORMATION

- Figure S1. MALDI-TOF mass spectra of 1  $\mu$ g human IgG digest (A) before enrichment and (B-D) after enrichment with different loading buffer by click maltose-HILIC tips. The enriched glycopeptides were marked with “ \* ”. (E) is the spectrum after on-column deglycosylation with 20  $\mu$ L 20mM  $\text{NH}_4\text{HCO}_3$  containing 100 U PNGase F for 30 min.
- Figure S2. Reproducibility of the reactor. The presented MALDI-TOF spectra were analysis of glycopeptides from three repeat processings of 1 $\mu$ g human IgG by the reactor. The glycopeptides were eluted without deglycosylation.
- Figure S3. Sensitivity for analysis of glycopeptides in human IgG when conventional digestion was used. The enriched glycopeptides were marked with “ \* ”.
- Figure S4. MS/MS spectra of glycopeptides identified from 1  $\mu$ g five-glycoprotein mixture by using the micro-reactor based method.

Figure S1

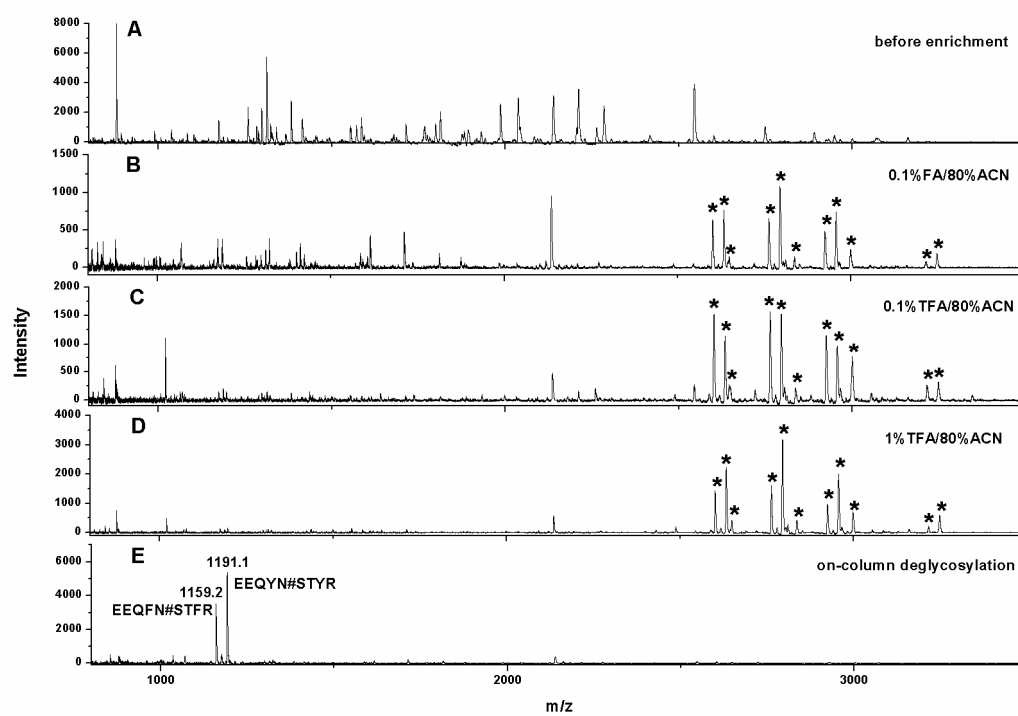


Figure S2

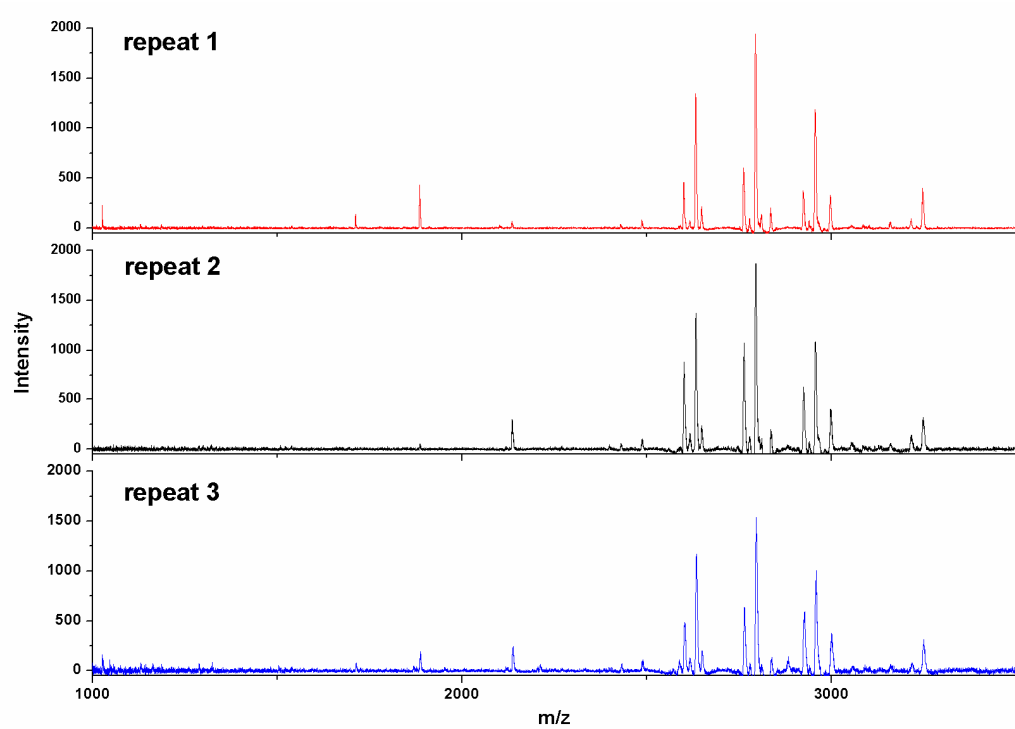


Figure S3

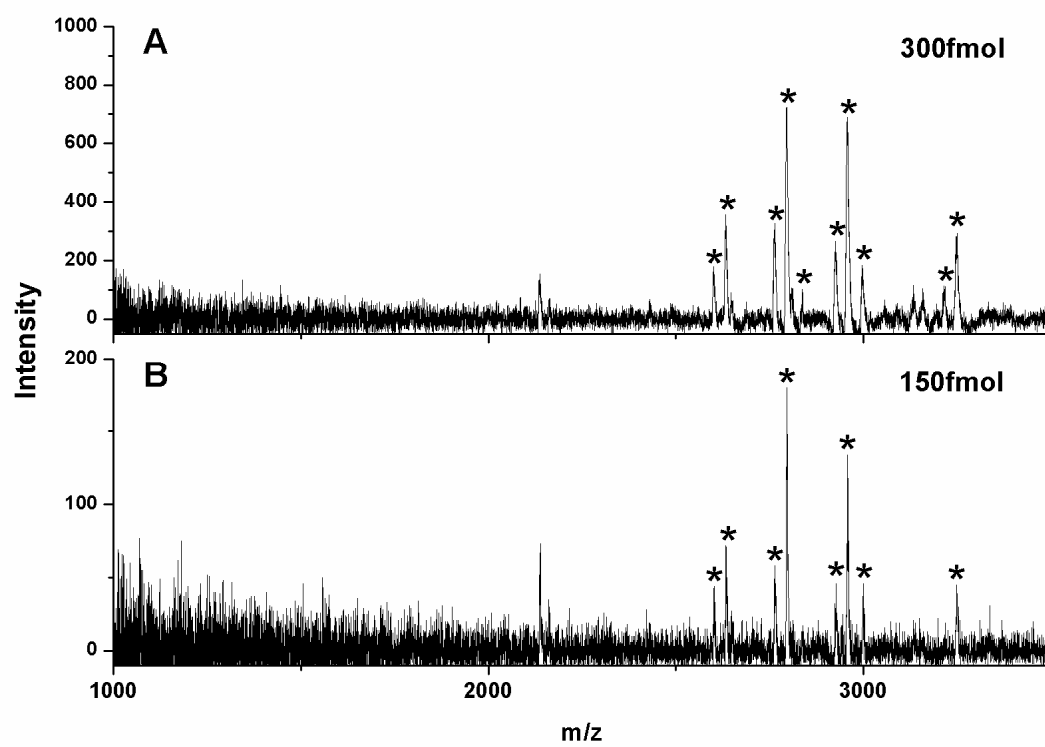
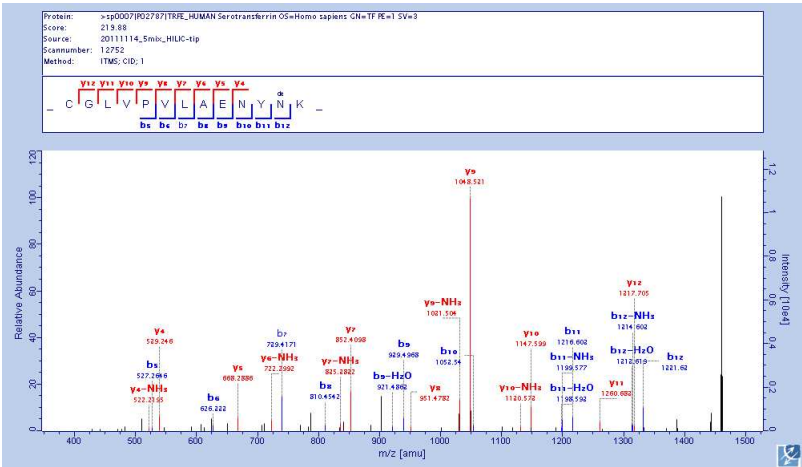
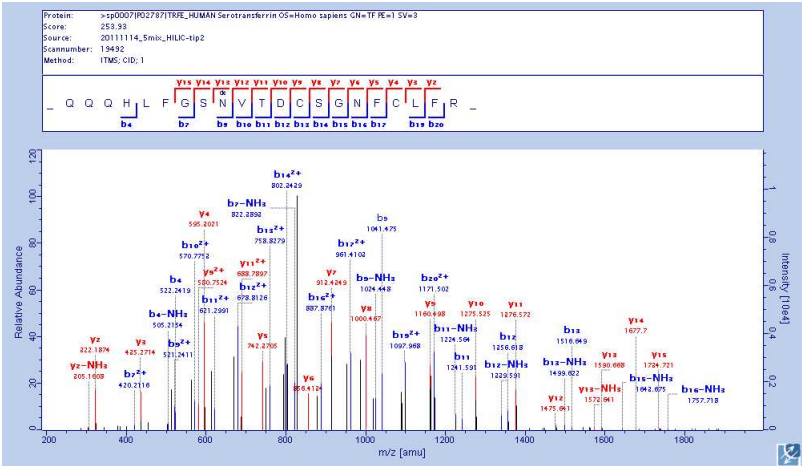


Figure S4

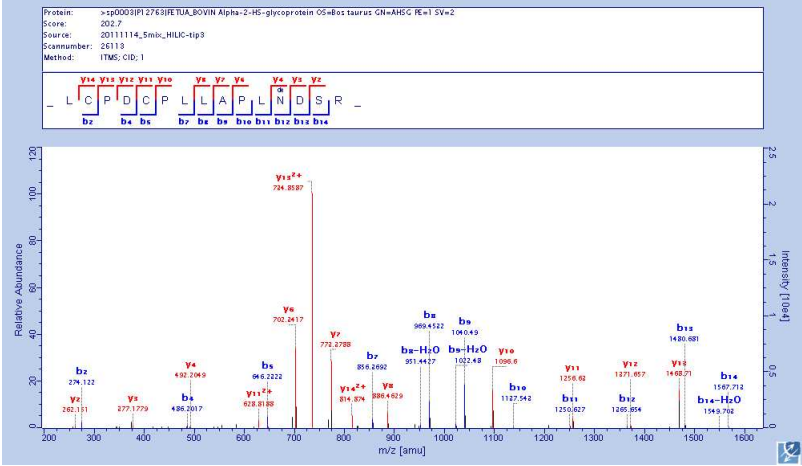
CGLVPVLAENY**N**K.S



QQQH**L**FGS**N**VTDCSGN**F**CLFR



LCPCDLLAP**L**NSR



[illegible][illegible]

Protein: s-sp000425SD421FE\_B0UVN Retain-B OS=bostrus GN=FE18B RE=2.5V=)

Score: 156.82

Source: 20111114\_5mbi\_n\_HLIC-tip2

Scannumber: 16100

Method: ITMS; CID: 1

**Sequence:** **- I F Y L P A Y N C T L R P V S Q S A I I M T C P D C P S T S P Y D L S N P R -**

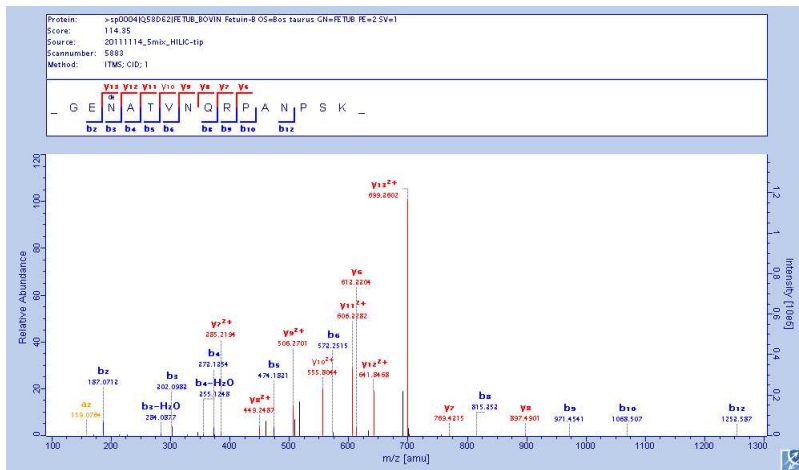
**Mass Spectrum:**

Relative Abundance vs. m/z [amu]

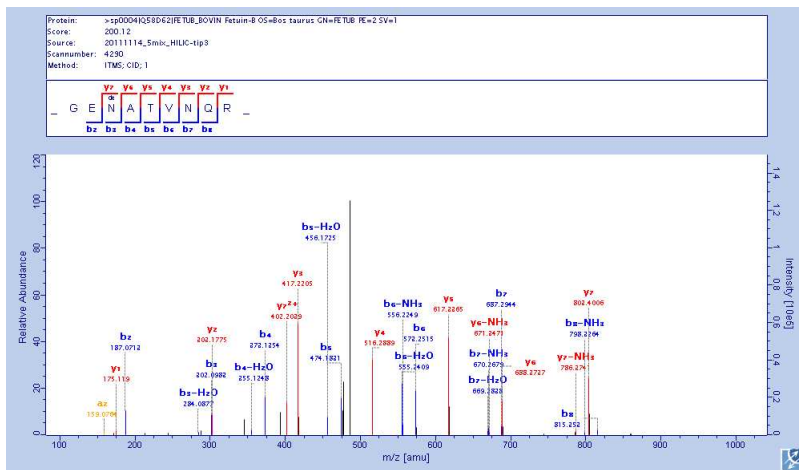
Key peaks labeled:

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- y<sub>1</sub>** 592.278
- y<sub>1</sub>+2** 922.292
- y<sub>1</sub>+4** 981.4728
- y<sub>1</sub>** 1045.509
- y<sub>1</sub>+2** 1065.50
- y<sub>1</sub>+4** 1085.51
- y<sub>1</sub>** 1165.979
- y<sub>1</sub>+2** 1185.97
- y<sub>1</sub>+4** 1226.586
- y<sub>1</sub>** 1351.106
- y<sub>1</sub>+2** 1371.294
- y<sub>1</sub>** 1447.162
- y<sub>1</sub>** 1492.469
- y<sub>1</sub>** 1512.751
- y<sub>1</sub>** 1531.754
- y<sub>1</sub>** 1551.754
- y<sub>1</sub>** 1571.754
- y<sub>1</sub>** 1591.754
- y<sub>1</sub>** 1611.754
- y<sub>1</sub>** 1631.754
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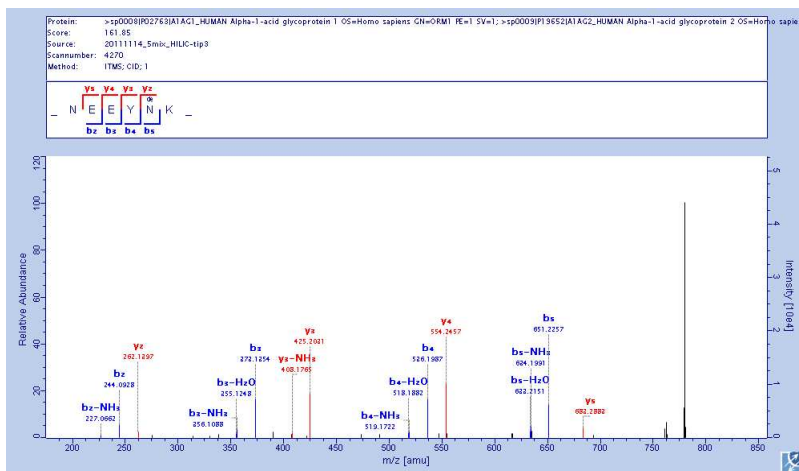
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GENATVNQR



NEEYNK.S





Protein: >sp000810|P02731|AT1G1.05.00 Homo sapiens cNOS-ORF1 [pe=1 SV1]

Score: 394.28

Source: 20111114\_5mhc\_HLUC-tlp3

Scan number: 13639

Method: ITMS; CID: 1

Y14	Y15	Y12	Y13	Y5	Y6	Y7	Y4	Y3	Y1	Y2
Q	D	Q	C	I	Y	N	T	T	Y	L
b5	b4	b5	b4	b7	b5	b10	b11	b12	b15	b14

Mass spectrum plot showing relative abundance versus m/z. The x-axis ranges from 300 to 1800 m/z, and the y-axis ranges from 0 to 120 relative abundance. Numerous peaks are labeled with their m/z values and corresponding ion types (e.g., Y4, b5-NH2, b7, b10, b11, b12, b15, b14). The base peak is at m/z 1006.232 (b7). Other significant peaks include m/z 1107.441 (b5-H2O), 1272.622 (Y10), and 1597.006 (b10-NH2).

Protein: >sp0081P00273J[ATAC]\_HUMAN Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 IE=1 SV=1

Score: 125.21

Source: 20111114\_5mhc\_HUIC-4ip3

Scannumber: 13559

Method: ITMS; CID: 1

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**Q D Q C I Y N T T Y L N V Q R E N G T I S R**

**b1 b2 b3 b4 b5 b6 b7 b8 b9 b10 b11 b12 b13 b14 b15 b16 b17 b18 b19 b20 b21 b22 b23 b24 b25 b26 b27 b28 b29 b30 b31 b32 b33 b34 b35 b36 b37 b38 b39 b40 b41 b42 b43 b44 b45 b46 b47 b48 b49 b50 b51 b52 b53 b54 b55 b56 b57 b58 b59 b60 b61 b62 b63 b64 b65 b66 b67 b68 b69 b70 b71 b72 b73 b74 b75 b76 b77 b78 b79 b80 b81 b82 b83 b84 b85 b86 b87 b88 b89 b90 b91 b92 b93 b94 b95 b96 b97 b98 b99 b100**

**bs bs-H<sub>2</sub>O bs-NH<sub>2</sub> Ys Ys-H<sub>2</sub>O Ys-NH<sub>2</sub> Ys<sup>2+</sup> Ys<sup>3+</sup> Ys<sup>4+</sup> Ys<sup>5+</sup> Ys<sup>6+</sup> Ys<sup>7+</sup> Ys<sup>8+</sup> Ys<sup>9+</sup> Ys<sup>10+</sup> Ys<sup>11+</sup> Ys<sup>12+</sup> Ys<sup>13+</sup> Ys<sup>14+</sup> Ys<sup>15+</sup> Ys<sup>16+</sup> Ys<sup>17+</sup> Ys<sup>18+</sup> Ys<sup>19+</sup> Ys<sup>20+</sup> Ys<sup>21+</sup> Ys<sup>22+</sup> Ys<sup>23+</sup> Ys<sup>24+</sup> Ys<sup>25+</sup> Ys<sup>26+</sup> Ys<sup>27+</sup> Ys<sup>28+</sup> Ys<sup>29+</sup> Ys<sup>30+</sup> Ys<sup>31+</sup> Ys<sup>32+</sup> Ys<sup>33+</sup> Ys<sup>34+</sup> Ys<sup>35+</sup> Ys<sup>36+</sup> Ys<sup>37+</sup> Ys<sup>38+</sup> Ys<sup>39+</sup> Ys<sup>40+</sup> Ys<sup>41+</sup> Ys<sup>42+</sup> Ys<sup>43+</sup> Ys<sup>44+</sup> Ys<sup>45+</sup> Ys<sup>46+</sup> Ys<sup>47+</sup> Ys<sup>48+</sup> Ys<sup>49+</sup> Ys<sup>50+</sup> Ys<sup>51+</sup> Ys<sup>52+</sup> Ys<sup>53+</sup> Ys<sup>54+</sup> Ys<sup>55+</sup> Ys<sup>56+</sup> Ys<sup>57+</sup> Ys<sup>58+</sup> Ys<sup>59+</sup> Ys<sup>60+</sup> Ys<sup>61+</sup> Ys<sup>62+</sup> Ys<sup>63+</sup> Ys<sup>64+</sup> Ys<sup>65+</sup> Ys<sup>66+</sup> Ys<sup>67+</sup> Ys<sup>68+</sup> Ys<sup>69+</sup> Ys<sup>70+</sup> Ys<sup>71+</sup> Ys<sup>72+</sup> Ys<sup>73+</sup> Ys<sup>74+</sup> Ys<sup>75+</sup> Ys<sup>76+</sup> Ys<sup>77+</sup> Ys<sup>78+</sup> Ys<sup>79+</sup> Ys<sup>80+</sup> Ys<sup>81+</sup> Ys<sup>82+</sup> Ys<sup>83+</sup> Ys<sup>84+</sup> Ys<sup>85+</sup> Ys<sup>86+</sup> Ys<sup>87+</sup> Ys<sup>88+</sup> Ys<sup>89+</sup> Ys<sup>90+</sup> Ys<sup>91+</sup> Ys<sup>92+</sup> Ys<sup>93+</sup> Ys<sup>94+</sup> Ys<sup>95+</sup> Ys<sup>96+</sup> Ys<sup>97+</sup> Ys<sup>98+</sup> Ys<sup>99+</sup> Ys<sup>100+</sup>**

**bs bs-H<sub>2</sub>O bs-NH<sub>2</sub> Ys Ys-H<sub>2</sub>O Ys-NH<sub>2</sub> Ys<sup>2+</sup> Ys<sup>3+</sup> Ys<sup>4+</sup> Ys<sup>5+</sup> Ys<sup>6+</sup> Ys<sup>7+</sup> Ys<sup>8+</sup> Ys<sup>9+</sup> Ys<sup>10+</sup> Ys<sup>11+</sup> Ys<sup>12+</sup> Ys<sup>13+</sup> Ys<sup>14+</sup> Ys<sup>15+</sup> Ys<sup>16+</sup> Ys<sup>17+</sup> Ys<sup>18+</sup> Ys<sup>19+</sup> Ys<sup>20+</sup> Ys<sup>21+</sup> Ys<sup>22+</sup> Ys<sup>23+</sup> Ys<sup>24+</sup> Ys<sup>25+</sup> Ys<sup>26+</sup> Ys<sup>27+</sup> Ys<sup>28+</sup> Ys<sup>29+</sup> Ys<sup>30+</sup> Ys<sup>31+</sup> Ys<sup>32+</sup> Ys<sup>33+</sup> Ys<sup>34+</sup> Ys<sup>35+</sup> Ys<sup>36+</sup> Ys<sup>37+</sup> Ys<sup>38+</sup> Ys<sup>39+</sup> Ys<sup>40+</sup> Ys<sup>41+</sup> Ys<sup>42+</sup> Ys<sup>43+</sup> Ys<sup>44+</sup> Ys<sup>45+</sup> Ys<sup>46+</sup> Ys<sup>47+</sup> Ys<sup>48+</sup> Ys<sup>49+</sup> Ys<sup>50+</sup> Ys<sup>51+</sup> Ys<sup>52+</sup> Ys<sup>53+</sup> Ys<sup>54+</sup> Ys<sup>55+</sup> Ys<sup>56+</sup> Ys<sup>57+</sup> Ys<sup>58+</sup> Ys<sup>59+</sup> Ys<sup>60+</sup> Ys<sup>61+</sup> Ys<sup>62+</sup> Ys<sup>63+</sup> Ys<sup>64+</sup> Ys<sup>65+</sup> Ys<sup>66+</sup> Ys<sup>67+</sup> Ys<sup>68+</sup> Ys<sup>69+</sup> Ys<sup>70+</sup> Ys<sup>71+</sup> Ys<sup>72+</sup> Ys<sup>73+</sup> Ys<sup>74+</sup> Ys<sup>75+</sup> Ys<sup>76+</sup> Ys<sup>77+</sup> Ys<sup>78+</sup> Ys<sup>79+</sup> Ys<sup>80+</sup> Ys<sup>81+</sup> Ys<sup>82+</sup> Ys<sup>83+</sup> Ys<sup>84+</sup> Ys<sup>85+</sup> Ys<sup>86+</sup> Ys<sup>87+</sup> Ys<sup>88+</sup> Ys<sup>89+</sup> Ys<sup>90+</sup> Ys<sup>91+</sup> Ys<sup>92+</sup> Ys<sup>93+</sup> Ys<sup>94+</sup> Ys<sup>95+</sup> Ys<sup>96+</sup> Ys<sup>97+</sup> Ys<sup>98+</sup> Ys<sup>99+</sup> Ys<sup>100+</sup>**

**bs bs-H<sub>2</sub>O bs-NH<sub>2</sub> Ys Ys-H<sub>2</sub>O Ys-NH<sub>2</sub> Ys<sup>2+</sup> Ys<sup>3+</sup> Ys<sup>4+</sup> Ys<sup>5+</sup> Ys<sup>6+</sup> Ys<sup>7+</sup> Ys<sup>8+</sup> Ys<sup>9+</sup> Ys<sup>10+</sup> Ys<sup>11+</sup> Ys<sup>12+</sup> Ys<sup>13+</sup> Ys<sup>14+</sup> Ys<sup>15+</sup> Ys<sup>16+</sup> Ys<sup>17+</sup> Ys<sup>18+</sup> Ys<sup>19+</sup> Ys<sup>20+</sup> Ys<sup>21+</sup> Ys<sup>22+</sup> Ys<sup>23+</sup> Ys<sup>24+</sup> Ys<sup>25+</sup> Ys<sup>26+</sup> Ys<sup>27+</sup> Ys<sup>28+</sup> Ys<sup>29+</sup> Ys<sup>30+</sup> Ys<sup>31+</sup> Ys<sup>32+</sup> Ys<sup>33+</sup> Ys<sup>34+</sup> Ys<sup>35+</sup> Ys<sup>36+</sup> Ys<sup>37+</sup> Ys<sup>38+</sup> Ys<sup>39+</sup> Ys<sup>40+</sup> Ys<sup>41+</sup> Ys<sup>42+</sup> Ys<sup>43+</sup> Ys<sup>44+</sup> Ys<sup>45+</sup> Ys<sup>46+</sup> Ys<sup>47+</sup> Ys<sup>48+</sup> Ys<sup>49+</sup> Ys<sup>50+</sup> Ys<sup>51+</sup> Ys<sup>52+</sup> Ys<sup>53+</sup> Ys<sup>54+</sup> Ys<sup>55+</sup> Ys<sup>56+</sup> Ys<sup>57+</sup> Ys<sup>58+</sup> Ys<sup>59+</sup> Ys<sup>60+</sup> Ys<sup>61+</sup> Ys<sup>62+</sup> Ys<sup>63+</sup> Ys<sup>64+</sup> Ys<sup>65+</sup> Ys<sup>66+</sup> Ys<sup>67+</sup> Ys<sup>68+</sup> Ys<sup>69+</sup> Ys<sup>70+</sup> Ys<sup>71+</sup> Ys<sup>72+</sup> Ys<sup>73+</sup> Ys<sup>74+</sup> Ys<sup>75+</sup> Ys<sup>76+</sup> Ys<sup>77+</sup> Ys<sup>78+</sup> Ys<sup>79+</sup> Ys<sup>80+</sup> Ys<sup>81+</sup> Ys<sup>82+</sup> Ys<sup>83+</sup> Ys<sup>84+</sup> Ys<sup>85+</sup> Ys<sup>86+</sup> Ys<sup>87+</sup> Ys<sup>88+</sup> Ys<sup>89+</sup> Ys<sup>90+</sup> Ys<sup>91+</sup> Ys<sup>92+</sup> Ys<sup>93+</sup> Ys<sup>94+</sup> Ys<sup>95+</sup> Ys<sup>96+</sup> Ys<sup>97+</sup> Ys<sup>98+</sup> Ys<sup>99+</sup> Ys<sup>100+</sup>**

**bs bs-H<sub>2</sub>O bs-NH<sub>2</sub> Ys Ys-H<sub>2</sub>O Ys-NH<sub>2</sub> Ys<sup>2+</sup> Ys<sup>3+</sup> Ys<sup>4+</sup> Ys<sup>5+</sup> Ys<sup>6+</sup> Ys<sup>7+</sup> Ys<sup>8+</sup> Ys<sup>9+</sup> Ys<sup>10+</sup> Ys<sup>11+</sup> Ys<sup>12+</sup> Ys<sup>13+</sup> Ys<sup>14+</sup> Ys<sup>15+</sup> Ys<sup>16+</sup> Ys<sup>17+</sup> Ys<sup>18+</sup> Ys<sup>19+</sup> Ys<sup>20+</sup> Ys<sup>21+</</sup>**

Protein: >sp0008|P02763|A1AG1\_HUMAN Glycine-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 IE=1 SV=1. >sp0003|P13652|A1AG2\_HUMAN Glycine-2-acid glycoprotein 2 OS=Homo sapiens

Score: 161.85

Source: 20111114\_5mhc\_HLUC-tip3

Scan number: 4270

Method: ITMS; CID: 1

**Peptide Sequence:** N E E Y N K

**Mass Spectrum:**

m/z [amu]	Relative Abundance (%)	Fragmentation Type
227.0660	~10	bs-NH <sub>2</sub>
244.0628	~15	bs
260.1297	~25	Y2
256.1089	~10	bs-NH <sub>2</sub>
255.1248	~15	bs-H <sub>2</sub> O
272.1254	~20	bs
408.1795	~15	Y3
415.2041	~20	Y4
438.1882	~15	bs-NH <sub>2</sub>
518.1722	~10	bs-NH <sub>2</sub>
519.1722	~10	bs-NH <sub>2</sub>
526.1987	~15	bs
554.2457	~35	Y4
604.1991	~15	bs-NH <sub>2</sub>
602.2151	~15	bs-H <sub>2</sub> O
651.2257	~30	bs
682.2392	~10	Y5
770.0000	100	bs

Protein: >sp00091P1|A2AC2\_HUMAN Alpha-1-acid glycoprotein 1 OS=Homo sapiens GR=NM2 RE=1 SV=2  
 Score: 248.13  
 Source: 20111114\_5mbs\_HILIC-tp2  
 Scannumber: 11360  
 Method: ITMS;CID; 1

- N Q N Q C F Y I N S S Y L N V I Q R -  
 bs bz bs bs bs bs bs bs bs bs bs bs

Relative Abundance (0 to 120)  
 m/z [amu] (200 to 1600)

Labeled Peaks (m/z):  
 226.0822 (bs-NH<sub>2</sub>), 260.1775 (Y), 285.3136 (Ys-NH<sub>2</sub>), 306.3310 (Ys), 326.3585 (Ys-NH<sub>2</sub>), 402.2459 (Ys), 421.3085 (Ys-NH<sub>2</sub>), 440.2278 (Ys), 478.082 (Ys-NH<sub>2</sub>), 516.2895 (Ys), 609.2668 (bs), 629.2729 (Ys), 678.2668 (bs), 702.7877 (bs), 762.4230 (Ys), 794.7227 (Ys), 810.2424 (Ys), 875.6582 (Ys), 949.5002 (bs-NH<sub>2</sub>), 956.2567 (bs), 961.2237 (bs), 969.4758 (bs-NH<sub>2</sub>), 985.278 (bs-H<sub>2</sub>O), 1026.262 (bs-NH<sub>2</sub>), 1042.289 (bs), 1112.41 (bs), 1130.421 (bs), 1152.296 (bs-NH<sub>2</sub>), 1226.418 (bs), 1276.476 (bs-NH<sub>2</sub>), 1292.436 (bs), 1406.350 (bs), 1428.518 (bs-NH<sub>2</sub>), 1495.350 (bs), 1520.81 (bs)

Protein: >sp000391P13652:TAG2\_HUMAN Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 RE=1 SVW2

Score: 84.12

Source: 20111114\_5mk\_HILUC-tip3

Scannumber: 12687

Method: ITMS; CID: 1

**Sequence:**

Q N Q C F Y N S Y L N V Q R E N G T V S R

**Masses:**

b<sub>2</sub> b<sub>4</sub> b<sub>7</sub> b<sub>10</sub> b<sub>12</sub> b<sub>13</sub>

**Masses:**

y<sub>2</sub> y<sub>3</sub> y<sub>5</sub> y<sub>6</sub> y<sub>7</sub> y<sub>8</sub> y<sub>9</sub> y<sub>10</sub> y<sub>11</sub> y<sub>12</sub> y<sub>13</sub> y<sub>14</sub> y<sub>15</sub> y<sub>16</sub> y<sub>17</sub> y<sub>18</sub> y<sub>19</sub> y<sub>20</sub> y<sub>21</sub> y<sub>22</sub> y<sub>23</sub> y<sub>24</sub> y<sub>25</sub> y<sub>26</sub> y<sub>27</sub> y<sub>28</sub> y<sub>29</sub> y<sub>30</sub> y<sub>31</sub> y<sub>32</sub> y<sub>33</sub> y<sub>34</sub> y<sub>35</sub> y<sub>36</sub> y<sub>37</sub> y<sub>38</sub> y<sub>39</sub> y<sub>40</sub> y<sub>41</sub> y<sub>42</sub> y<sub>43</sub> y<sub>44</sub> y<sub>45</sub> y<sub>46</sub> y<sub>47</sub> y<sub>48</sub> y<sub>49</sub> y<sub>50</sub> y<sub>51</sub> y<sub>52</sub> y<sub>53</sub> y<sub>54</sub> y<sub>55</sub> y<sub>56</sub> y<sub>57</sub> y<sub>58</sub> y<sub>59</sub> y<sub>60</sub> y<sub>61</sub> y<sub>62</sub> y<sub>63</sub> y<sub>64</sub> y<sub>65</sub> y<sub>66</sub> y<sub>67</sub> y<sub>68</sub> y<sub>69</sub> 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Protein: xp00023101.85|IGHG1\_HUMAN Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 RE=1

Score: 129.03

Source: 20111114\_5mks\_HILUC-tip3

Scan number: 6953

Method: LTMS, CID: 1

Y <sup>6</sup>	Y <sup>7</sup>	Y <sup>8</sup>	Y <sup>9</sup>	Y <sup>4</sup>	Y <sup>2</sup>	Y <sup>5</sup>	Y <sup>1</sup>
E	Q	Y	N	S	T	Y	R
b <sub>2</sub>	b <sub>2</sub>	b <sub>4</sub>	b <sub>4</sub>	b <sub>2</sub>	b <sub>2</sub>	b <sub>2</sub>	

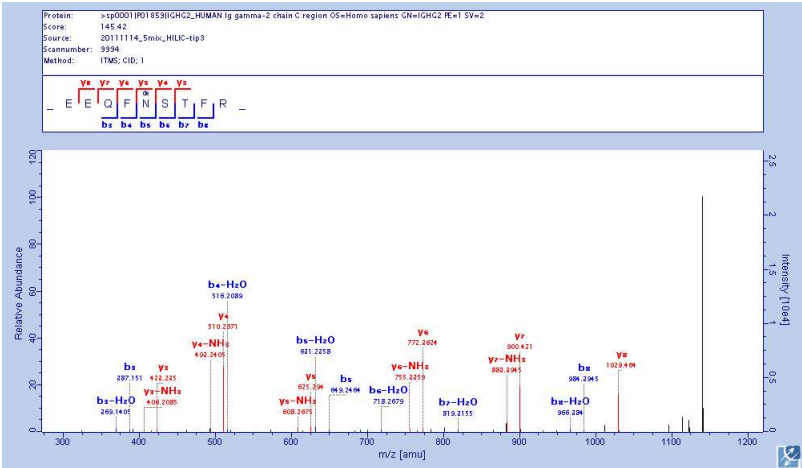
Relative Abundance

m/z [amu]

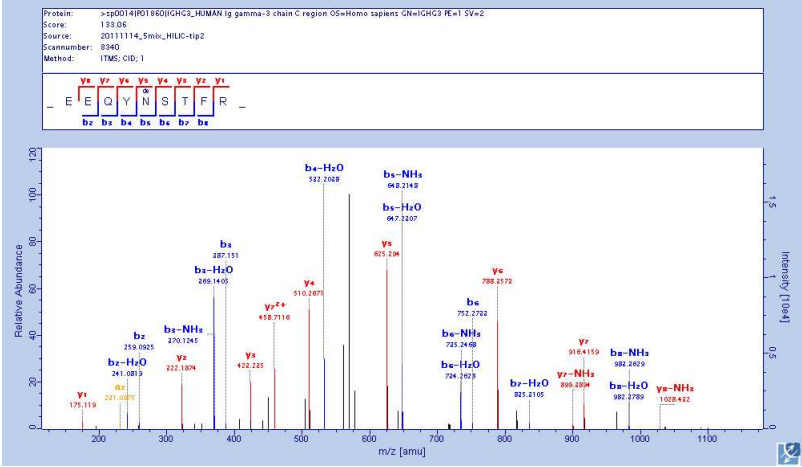
Peak Data:

m/z	Label
175.1119	Y1
195.1119	Y1
215.0921	b2
221.1557	Y2-NH3
228.1822	Y2
249.1405	b4-H2O
270.1245	b5-NH3
286.7091	Y4+H
395.1729	b4
398.1729	b4-H2O
420.22	Y3
522.2028	b4-H2O
526.1062	Y4
641.2089	Y1
647.2207	b5-H2O
721.248	b5-NH3
724.2628	b5-H2O
802.2105	b2-H2O
808.2522	Y6
909.0578	b4-NH3
912.4100	Y7-NH3
915.2361	Y7
95.221	b2
101.0124	b4
108.1452	Y6

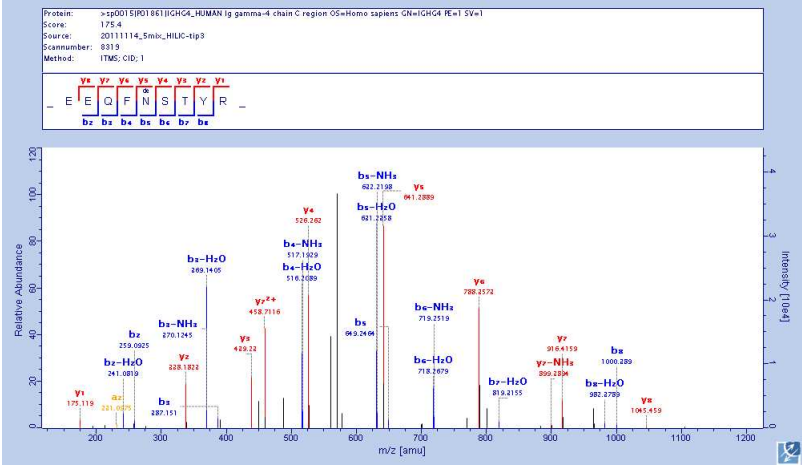
EEQFNSTFR



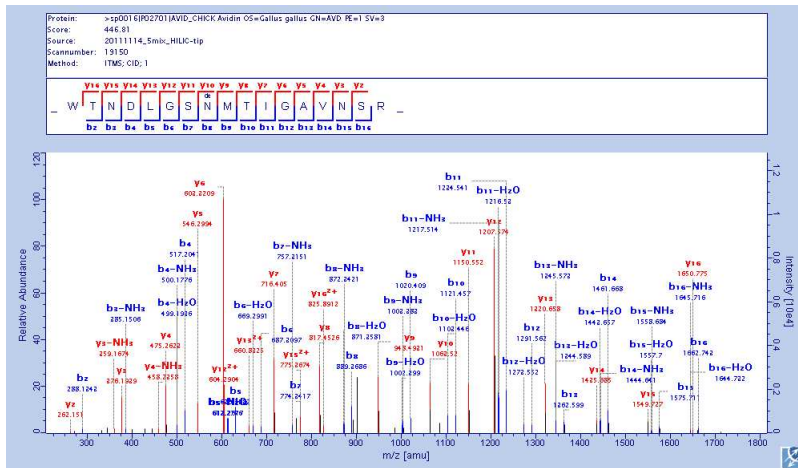
EEQYNSTFR



EEQFNSTYR



WTNDLGSNMTIGAVNSR

CSLTGKWTNDLGSNMTIGAVNSR