

# Application News

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# Biopharma / LCMS-9030 (Q-TOF)

# In-depth Peptide Mapping of Monoclonal Antibody (mAb) by A *de novo* Peptide Sequencing Method on Q-TOF Mass Spectrometer with Data-independent Acquisition

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# Introduction

Peptide mapping is an important and efficient technique for the primary structure characterization of monoclonal antibodies (mAbs). It involves enzymatic digestion (e.g., trypsin) and enables the direct detection of single amino acid changes. Also, it can provide additional information of post-translational modifications (PTMs). However, we have to note that peptide mapping is usually performed in a comparative manner, in which the peptide map of a mAb product is compared to that of a reference in a side-by-side experiment, which is highly dependent on the reproducible sample preparation and extremely time consuming. An in-depth peptide mapping workflow with a de novo sequencing method is thus required to verify primary structure of mAb and identify any alterations in structure. An emerging technique, and the focus of this report, is data-independent acquisition (DIA) mode on Q-TOF mass spectrometry. DIA enables a genetic, nonbiased approach for the MS/MS data acquisition, in which all the ions in each individual MS/MS event are fragmented without precursor ion isolation and all the fragment ions are measured in a mixed spectrum, ideal for peptide sequencing. In this report, we demonstrated an integrated MS full-scan and MS/MS DIA approach LCMS<sup>™</sup>-9030 on Shimadzu (Q-TOF) mass spectrometer for de novo peptide sequencing of mAb (Figure 1).

## Experimental

A 5 mg/mL of bevacizumab biosimilar sample solution was prepared in 50 mmol/L Tris-HCI (pH 8.0) buffer. A 20  $\mu$ L aliquot of the sample was diluted with 80  $\mu$ L of ammonium bicarbonate (ABC) solution (50 mM), then mixed with 10  $\mu$ L ProteaseMAX<sup>TM</sup> (0.5%, w/w) and 10  $\mu$ L Dithiothreitol (DTT, 0.2 M), incubated at 60°C for 60 min to denature and reduce disulfide bonds. Alkylation was done by adding 30  $\mu$ L iodoacetamide (IAM, 0.2 M) followed by incubation at 37°C for 60 min in the dark. The sample were diluted with 328  $\mu$ L ABC solution (50 mM) before trypsin digestion. Sequencing grade trypsin was used for digestion at 37°C for overnight. Finally, 2  $\mu$ L trifluoroacetic acid (TFA) was added to stop activity of trypsin. The sample was centrifuged and supernatant was collected and injected to LCMS-9030 (Q-TOF) for

DIA analysis. The analytical conditions are displayed in **Table 1**, and the MS and MS/MS segments is shown in **Table 2** 

| Table 1. Analytica | conditions on | LCMS-9030 (Q-TOF) |
|--------------------|---------------|-------------------|
|--------------------|---------------|-------------------|

| LC conditions           |  |
|-------------------------|--|
| LC system:              | Shimadzu Nexera™ X2 UHPLC                                      |
| Column:                 | Shim-pack™ GISS-HP, 3 µm, 150 × 3.0                            |
| Column temperature:     | mm 40 °C   |
| Flow rate:              | 0.5 mL/min   |
| Mobile phase A:         | 0.1% FA + 0.01% TFA in water                                   |
| Mobile phase B:         | 0.1% FA + 0.01% TFA in acetonitrile                            |
| Gradient program:       | B Conc. 0% (0-2 min) $\rightarrow$ 15% (10 min) $\rightarrow$  |
|                         | 35% (23 min) $\rightarrow$ 45% (30 min) $\rightarrow$ 75% (35- |
|                         | 40 min) $\rightarrow$ 0% (40.1-45 min).                        |
| Injection volume:       | 20 µL  |
|                         |  |
| MS conditions           |  |
| MS system:              | Shimadzu LCMS-9030 (QTOF)                                      |
| Interface:              | Heated ESI (+)   |
| Interface voltage:      | 4.5 kV   |
| Interface temperature:  | 300 °C   |
| Nebulizing gas:         | N2, 3 L/min  |
| Heating gas flow:       | Zero air, 10L/min  |
| DL temperature:         | 250 °C   |
| Drying gas flow:        | N2, 10 L/min   |
| Heat block temperature: | 400 °C   |
| MS mode:                | MS   |
| Mass range:             | 100 - 2000 m/z   |
| MS/MS mode:             | DIA  |
| Mass segment:           | From 210 to 1690 by 40 m/z                                     |
| Collision Energies:     | 35 ± 17 V  |

## Results and Discussion

### A. MS and MS/MS data extraction

The raw MS data file (.lcd) was exported as .mzML data by LabSolutions<sup>TM</sup> s/w, and then uploaded into MS-DIAL s/w [2] for both MS and MS/MS data extraction. The default parameters in MS-DIAL were applied for peak detection and extraction with the minimum peak height setting at 1500 amplitude. Finally, 11,852 mass peaks including the information of precursor *m*/*z*, retention time, peak height, adduct ions, and MS/MS fragment ions were extracted (data not shown).



Figure 1. The *de novo* peptide sequencing approach on *LCMS-9030 (Q-TOF)* for characterization of mAb. The peptide ALPAPIEK was used as an example for illustration.

|            |         | •       |         |
|------------|---------|---------|---------|
| Experiment | MS Type | Min m/z | Max m/z |
| 0          | MS      | 100     | 2000    |
| 1          | MS/MS   | 210     | 250     |
| 2          | MS/MS   | 250     | 290     |
| 3          | MS/MS   | 290     | 330     |
| 4          | MS/MS   | 330     | 370     |
| 5          | MS/MS   | 370     | 410     |
| 6          | MS/MS   | 410     | 450     |
| 7          | MS/MS   | 450     | 490     |
| 8          | MS/MS   | 490     | 530     |
| 9          | MS/MS   | 530     | 570     |
| 10         | MS/MS   | 570     | 610     |
| 11         | MS/MS   | 610     | 650     |
| 12         | MS/MS   | 650     | 690     |
|            |         |         |         |
|            |         |         |         |
|            |         |         |         |
| 30         | MS/MS   | 1370    | 1410    |
| 31         | MS/MS   | 1410    | 1450    |
| 32         | MS/MS   | 1450    | 1490    |
| 33         | MS/MS   | 1490    | 1530    |
| 34         | MS/MS   | 1530    | 1570    |
| 35         | MS/MS   | 1570    | 1610    |
| 36         | MS/MS   | 1610    | 1650    |
| 37         | MS/MS   | 1650    | 1690    |

Table 2, MS and MS/MS experiment file

#### B. Theoretical MS/MS transitions of peptides

The amino acid sequence of bevacizumab downloaded from DrugBank <u>https://www.drugbank.ca</u> was imported into Skyline s/w [3] for MS/MS transitions prediction. In peptide settings, tryptic peptides with carbamidomethyl modification were selected. In transition settings, only singly charged y and b ions (+) were predicted, as they are the most common fragmentations observed in low energy collisions.

#### C. Peptide sequencing

The main aim of this study is to explore the potential of MS/MS DIA method for in-depth peptide mapping at *de novo* peptide sequencing level. Based on our previous application news of AD-0212B [1], 42 and 19 peptides were measured from the heavy-chain and light-chain respectively, covering 100% amino acid sequence of bevacizumab biosimilar. In the present report, we focus on the same peptides for *de novo* peptide sequencing. By matching the extracted MS/MS spectra data by MS-DIAL to the theoretical MS/MS transitions of tryptic peptide of bevacizumab in Skyline, all the 61 peptides were well identified and verified for their amino acid sequences (**Tables 3 and 4**).

### Table 3. De novo peptide sequencing of bevacizumab biosimilar heavy chain by MS/MS DIA approach on LCMS-9030

| No.     Lyber productory     (mon all productor)       4001     -PUALVESCODUVOPCOSER L[1:19]     91.5669     11.5699     (mon all productor)     (mon all productor   | Peak         | Pentide [AA numbers]  | MS            | RT                    | MS/MS   |
|---|--------------|---|---------------|-----------------------|---|
| Horis     - EVQLVESCOLUCPOOBLE [L]     H1 5935-41       H1 5935-41     H1 5935   | No.          | r epilde [AA humbers]   | (m/z&z, mea.) | (min)                 | (m/z, mea.) matched with predicted transitions in Skyline s/w   |
| Incur     Responses/memory/memory/section     Part Park (P): 1987 998 (P): 1987 998 (P): 1985 298 (P): 1985             | HC01         | EVQLVESGGGLVQPGGSLR.L [1, 19]   | 941.5052++    | 17.23                 | 1313.6813 (y14); 1184.6338 (y13); 1097.6059 (y12); 1040.5843 (y11); 983.6615 (y10); 926.5446 (y9);<br>813.4589 (y8); 714.3893 (y7); 586.3310 (y6); 489.2777 (y5); 432.2592 (y4); 375.2331 (y3); 288.2014<br>(y2); 175.1183 (y1); 229.1159 (b2); 357.1755 (b3); 470.2602 (b4); 569.3301 (b5); 698.3733 (b6);<br>785.4051 (b7); 842.4365 (b8); 899.4452 (b9); 956.4699 (b10); 1069.5449 (b11); 1168.6183 (b12);<br>1296.6757 (b13)  |
| LCDD     ALORENG DIR, ALI     DOLEND     LCD     DOLEND ALORENG DIR, LCD  | HC02         | R.LS <b>C</b> AASGYTFTNYGMNWVR.Q [20, 38]   | 1099.4940++   | 20.67                 | 1608.7324 (y13); 1287.5968 (y10); 1140.5254 (y9); 925.4361 (y7); 762.3740 (y6); 574.3081 (y4);  |
| No.     K.OLEWYGWNYTYDE/TYAADFK R[44].     MED/2000 [17]     M  | HC03         | R.QAPGK.G [39. 43]  | 500.2829+     | 3.50                  | 204.1326 (y2)   |
| Liss     Access (rs), responses     State (rs), responses <thstate (rs),="" responses<="" th="">     State (rs),</thstate>  | HC04         | K.GLEWVGWINTYTGEPTYAADFK.R [44,<br>65]  | 840.0690+++   | 24.13                 | 1690.7969 (y15); 1577.7154 (y14); 1463.6656 (y13); 1362.6230 (y12); 1199.5587 (y11); 1098.5108 (y10); 1041.4901 (y9); 912.4464 (y8); 815.3941 (y7); 714.3451 (y6); 551.2815 (y5); 480.2456 (y4); 409.2080 (y3); 294.1815 (y2); 147.1125 (y1); 171.1124 (b2); 300.1548 (b3); 486.2344 (b4); 585.3038 (b5); 642.3237 (b6); 828.4038 (b7); 941.4867 (b8); 1055.5297 (b9); 1156.5748 (b10); 1319.6389 (b11); 1420.6792 (b12); 1477.7128 (b13); 1606.7544 (b14)  |
| Line     Bit Instead 10     Part 18 (10)       1000     REAR Park     Park 18 (10)       1000     REAR Park     Park 18 (10)       1000     REAR Park LTSKS (ER, 78) (Instead 1)     Park 18 (10)       1000     REAR Park LTSKS (ER, 78) (Instead 1)     Park 18 (10)       1000     REAR Park LTSKS (ER, 78) (Instead 1)     Park 18 (10)       1000     REAR Park LTSKS (ER, 78) (Instead 1)     Park 18 (10)       1000     REAR Park LTSKS (ER, 78)     Park 18 (10)     Park 18 (10)       1000     REAR Park LTSKS (ER, 78)     Park 18 (10)     Park 18 (10)     Park 18 (10)       1000     REAR Park LTSK (ER, 78)     Park 18 (10)     Park   | HC38         | K.GLEWVGWINTYTGEPTYAADFKR.R [44,  | 892.1030+++   | 23.00                 | 1355.6526 (y12); 1068.5361 (y9); 450.2716 (y3); 171.1125 (b2); 300.1551 (b3); 642.3293 (b7);  |
| 1036.5271 (yb)     1036.57  | HC39         | 66] (missed 1)<br>K RR F [66, 67] (missed 1)  | 331 2198+     | 2.03                  | 941.4961 (08)<br>175.1195 (v1)  |
| HODE     ENTISECTER & [B0, 76]     S22,2842++     Total     Biol 4211 (bit 774441 (bit 26012), Biol 1200 (bit 2003 (bit 100), Biol 1200 (bit 2003), Biol 12000, Bio                                       | HC40         | R.RFTFSLDTSK.S [67, 76] (missed 1)  | 601.3149++    | 16.40                 | 1045.5218 (y9); 898.4441 (y8); 797.4053 (y7); 650.3378 (y6); 563.3060 (y5); 450.2189 (y4); 335.1922 (y3); 234.1444 (y2); 147.1124 (y1); 157.1072 (b1); 304.1762 (b2); 405.2223 (b3); 552.2912 (b4); 639.3256 (b5); 752.4083 (b6); 867.4357 (b7); 968.4825 (b8); 1055.5141 (b9)  |
| Hote     1005     5511 (pd), 104, 2021 (pd), 99, 91, 911 (pd), 714,379 (pd), 2021 78 (pd), 718 (p             | HC05         | R.FTFSLDTSK.S [68, 76]  | 523.2642++    | 17.40                 | 898.4521 (y8); 797.4041 (y7); 650.3358 (y6); 563.3035 (y5); 450.2190 (y4); 335.1921 (y3); 234.1446  |
| Home     1219:5344 (r/m): 1004-677 (r/m): 754-5802 (s): 784-478 (s): 703.377 (r/m): 714 (r/m): 201008 (s): 716 (r/m): | HC06         | K.STAYLQMNSLR.A [77, 87]  | 642.3186++    | 15.97                 | 1095.5611 (y9); 1024.5251 (y8); 861.4611 (y7); 748.3765 (y6); 620.3179 (y5); 489.2774 (y4);<br>375.2346 (y3); 288.2020 (y2); 175.1186 (y1); 189.0862 (b2); 260.1238 (b3); 423.1864 (b4);<br>536.2715(b5); 664.3289 (b6); 795.3706 (b7); 909.4114 (b8); 996.4463 (b9); 1109.5303 (b10)   |
| HC08     KVPHYVDSBRVVFDWGGGTLVTVSSA<br>STK.G [198 127]     1108.5200+H     Z100     200.201   | HC07         | R.AEDTAVYY <b>C</b> AK.Y [88, 98]   | 645.7824++    | 12.43                 | 1219.5344 (y10); 1090.4877 (y9); 975.4592 (y8); 874.4123 (y7); 803.3761 (y6); 704.3072 (y5);<br>541.2430 (y4); 378.1801 (y3); 218.1495 (y2); 147.1121 (y1); 201.0865 (b2); 316.1142 (b3); 417.1609<br>(b4); 488.1986 (b5); 587.2665 (b6); 750.3321 (b7); 913.3932 (b8); 1073.4188 (b9); 1144.4664 (b10)   |
| HO2     KGPSVFFLAPSKS [128, 139]     503.820+**     1028-272 (v)0; 464.536 (v)0; 124.123 (v)1; 120 (v)2; 121 (128 (v)); 120, 122 (v)1; 100.366 (v)0; 120, 121 (128 (v)); 120, 120 (v)1;                           | HC08         | K.YPHYYGSSHWYFDVWGQGTLVTVSSA<br>STK.G [99, 127]   | 1108.5200+++  | 22.00                 | 1521.7817 (y15); 1150.6364 (y12); 992.5611 (y10); 879.4779 (y9); 780.4088 (y8); 679.3571 (y7);<br>580.2946 (y6); 493.2662 (y5); 335.1915 (y3); 147.1111 (y1); 261.1220 (b2); 398.1855 (b3); 561.2379<br>(b4); 1802.7533 (b14)   |
| HC10     K.ST3GGTAALOQLIVK D [140, 153]     661 3355++     153 (301) (712) (104, 586, 197) (303, 193) (901) (317 (2)), (171, 122 (7)), (190, 683, 198) (90);       K.ST3GGTAALOQLIVK D [140, 153]     661 3355++     156 (313, 304, 91), 305 (91) (330, 301, 93) (91) (317 (2), 71, 1122 (7)); (190, 683, 198) (90);       K.DYFPEPUT/SWMSQALTSGVTFPAVL     679, 0850+++++, 427     243, 3111 (02); 177, 288, 307 (90);     243, 3111 (02); 177, 288, 307 (90);     243, 3111 (02); 177, 288, 307 (90);     243, 3111 (02); 177, 1288, 3131 (02); 171, 126, 310;     243, 3161 (02); 177, 1288, 311 (02); 171, 226, 315 (02);     243, 3111 (02); 177, 1288, 3121 (02); 123, 3138 (04);     243, 3131 (02); 177, 1288, 3131 (02); 171, 226, 313, 3164 (03);       ICC1     KUDKK [17, 219]     361, 2089 -     21, 33     390, 2335 (91); 275, 273 (92); 147, 1124 (91); 124, 1012 (02); 33, 1384 (03);       ICC1     KVDKK [21, 22]     164     483, 200, 17, 124, 592     21, 277, 696     22, 275, 273 (91); 171, 171, 264 (91); 171, 171, 264 (91); 171, 171, 191, 191, 191, 191, 191, 191,  | HC09         | K.GPSVFPLAPSSK.S [128, 139]   | 593.8290++    | 17.10                 | 1032.5725 (y10); 945.5395 (y9); 846.4723 (y8); 699.4035 (y7); 602.3510 (y6); 489.2650 (y5);<br>418.2294 (y4); 321.1758 (y3); 234.1445 (y2); 147.1128 (y1); 155.0812 (b2); 242.1131 (b3); 341.1816<br>(b4); 488.2507 (b5); 698.3879 (b7); 769.4247 (b8); 953.5011 (b10); 1040.5395 (b11)   |
| HC175     K-DYPEP-VTX9MNGALTSQ/HTPRAL     223331811 (u22): 1075 3020 (r/4); 1228.02210 (u3); 278.0775 (u2); 24.8657 (db); 221.6654 (h2); 222.6654 (h2); 222.6763 (h2);              | HC10         | K.STSGGTAALG <b>C</b> LVK.D [140, 153]  | 661.3535++    | 15.60                 | 1133.6011 (y12); 1046.5643 (y11); 989.5407 (y10); 831.4741 (y8); 760.4365 (y7); 689.3999 (y6);<br>576.3168 (y5); 519.2965 (y4); 359.2633 (y3); 246.1812 (y2); 147.1122 (y1); 189.0866 (b2); 276.1187<br>(b3); 333.1410 (b4); 390.1581 (b5); 803.3955 (b10)  |
| HC12     KVDK (217, 219)     381 2089     2.63     382 1394 (p2): 147.1124 (p1): 215.1014 (b2): 343.1984 (b3)       HC44     KVDKK V217, 220 (missed 1)     600.3716+     4.50     422 2757 (p4): 373 2072 (p3): 244.1684 (p1): 249.1020 (p1): 228.1020 (p2): 357.2127       HC14     KVDEVK S127.02 (24) (missed 1)     600.3716+     4.50     432 2007 (p3): 244.1684 (p1): 243.087 (p1): 243.0864 (p3)       HC14     KSQEXT 1223, 2281     900.2029+     2.20     1393.092 (147): 1147 (p1): 243.087 (p1): 1337.07381 (p4): 1151.7612       HC15     K.THC2PCPAPELLGGPSVFLFPPKPKD     948.8240+++     21.93     (p6): 1563.562 (p4): 973.25382 (p3): 832.694 (p3): 1533.7614 (p3): 1147.1680 (p1): 1147.1653 (p1): 1145.358 (p1): 1145.155 (p1): 1147.1553 (p1): 1145.358 (p1): 1145.155 (p1): 1147.1553 (p1): 1145.358 (p1): 1145.155 (p1): 1145.155 (p1): 1147.1553 (p1): 1145.358 (p1): 1145.155 (p1): 1147.1553 (p1): 1145.1553 (p2): 1145.1553 (p2): 1144.1553 (p1): 1145.   | HC11         | K.DYFPEPVTVSWNSGALTSGVHTFPAVL<br>QSSGLYSLSSVVTVPSSSLGTQTYI <u>C</u> NVN<br>HKPSNTK.V [154, 216] | 1679.0850++++ | 24.97                 | 2433.1811 (y22); 1675.8302 (y14;); 1298.6312 (y11); 925.4857 (y8); 811.4421 (y7); 674.3846 (y6); 546.2880 (y5); 449.2345 (y4); 362.2010 (y3); 279.0973 (b2); 426.1657 (b3); 523.2169 (b4); 652.2640 (b5); 749.3157 (b6); 848.3809 (b7); 949.4295 (b8); 1048.5006 (b9); 1135.5261 (b10); 1321.6154 (b11)   |
| Heat I, KUDKKV [217, 220] (Imissed 1)     489-3309     [213]     390-2356 (191, 274, 2764 (192), 284, 1684 (192), 147, 1126 (111), 228, 1692 (102), 287, 2127       Heat K, KVEPKS [220, 220]     490-3764     497-2278 (191, 284, 1685 (192), 124, 1126 (111), 228, 1696 (102), 287, 2127       Heat K, KVEPKS [221, 228]     509-2029+     2.20     227, 207, 201, 201, 201, 201, 201, 201, 201, 201  | HC12         | K.VDK.K [217, 219]  | 361.2089+     | 2.53                  | 262.1394 (y2); 147.1124 (y1); 215.1020 (b2)   |
| HC42     KKVEPK S120. 224 (missed 1)     600.31/6     4.60     003.164     4.60     003.164     4.60     003.164     4.60     003.164     4.61     KUEPK S120.224     403.22764     520.272     473.207 (3)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)     473.207 (4)   | HC41         | K.VDKK.V [217, 220] (missed 1)  | 489.3030+     | 2.13                  | 390.2335 (y3); 275.2073 (y2); 147.1124 (y1); 215.1014 (b2); 343.1984 (b3)<br>472 2757 (y4): 373 2072 (y3): 244 1648 (y2): 147 1128 (y1): 129 1020 (b1): 228 1692 (b2): 357 2127   |
| HC13     KVEPK, S[221, 224]     472.2766+     5.20     373.2067 (x); 244.1651 (x); 147.1121 (x); 248.0667 (b2); 363.0694 (b3)       HC14     K SQDKT [225, 228]     509.2029+     2.0     422.1701 (x); 225.2281 (x); 147.1121 (x); 248.0667 (b2); 363.0694 (b3)       HC15     K SQDKT [225, 228]     509.2029+     2.0     422.1701 (x); 225.2264 (x); 244.1684 (y); 147.1121 (x); 248.0667 (b1); 353.0698 (b1); 123.17612 (y); 223.2561 (x); 244.1684 (y); 147.1121 (y); 238.1136 (b2); 243.1134 (b2); 340.1603 (b3) (b1) 115 (b362 (b1)); 153.6886 (y); 827.6328 (b3); 153.2571 (y); 237.1278 (y); 327.1278 (y); 327.12   | HC42         | K.KVEPK.S [220, 224] (missed 1)   | 600.3716+     | 4.50                  | (b3); 454.2650 (b4)   |
| HC14     KSUK [122, 22]     509.2091     220     Pach T0 (19), 112 (12), 100, 124, 200, 200, 200, 200, 200, 200, 200, 2   | HC13         | K.VEPK.S [221, 224]   | 472.2766+     | 5.20                  | 373.2067 (y3); 244.1651 (y2); 147.1124 (y1); 229.1180 (b2)  |
| HC16     K.DTLMISR.T [255, 261]     418.2220++     13.27     506.2756 (w); 372.217 (w); 327, 1185 (w); 217, 2021 (w); 330, 1249 (w);       HC17     R.TPEVT©VVVDVSHEDPEVKF [262, 280]     713.6810+++     17.27     517, 328 (w);     573.326 (x);     573.326 (x);     573.326 (x);     577.57 (w);     523.5201 (w);     243.536 (w);     043.666 (w);       HC17     R.TPEVT©VVDVSHEDPEVKF [262, 280]     713.6810+++     17.27     171.3846 (w);     957.3052 (w);     377.577 (w);     523.655 (w);     304.0466 (w);     985.5012 (w);     105.266 (b10);     1196.046 (b11);     111.226 (c11);     1102.266 (b10);     1196.046 (b11);     112.27 (w);     585.2582 (w);     585.3126 (w);     687.3326 (w);     987.3126 (w);     987.326 (w);   | HC14         | K.THT <u>C</u> PPCPAPELLGGPSVFLFPPKPK.D<br>[229, 254]   | 948.8240+++   | 21.93                 | 422.1101 (3), 202.135 (32), 147.1121 (31), 240.037 (34), 300.3394 (37), 7831 (31), 7612 (37), 7831 |
| HC17     R.TPEVTQVVDVSHEDPEVKF [262, 280]     713.6810+++     172     F13.33 (r13); 132.271 (r13.346 (r6); 567.303 (r6); r127.253.601 (r10); r126.4358 (r0); r032.603 (r6); r227.275 (r10); r226.612 (r10); r126.633 (r10); r226.612 (r10); r226.61                                       | HC16         | K.DTLMISR.T [255, 261]  | 418.2220++    | 13.27                 | 506.2759 (y4); 375.2371 (y3); 262.1507 (y2); 175.1185 (y1); 217.0821 (b2); 330.1649 (b3)  |
| HC18     K.FNWYVDGVEVHNAK.T [281, 294]     839.3967++     17.67       MC18     K.FNWYVDGVEVHNAK.T [281, 294]     839.3967++     17.67       MC18     K.FNWYVDGVEVHNAK.T [281, 294]     839.3967++     17.67       MC18     K.FNWYVDGVEVHNAK.T [281, 294]     839.3967++     17.67       MC20     R.EEQYNSTYR.V [299, 307]     595.2588++     10.20       MC21     R.EEQYNSTYR.V [299, 307]     595.2588++     10.20       MC21     R.EEQYNSTYR.V [299, 307]     595.2588++     10.20     803.3704 (y6): 640.3070 (y5); 526.2682 (y4); 439.2217 (y3)       HC21     R.VVSVLTVLHODWUNGK E [308, 323]     94.5058++     10.20     803.3704 (y6): 640.3070 (y5); 526.2682 (y4); 439.2217 (y3)       HC22     KEYK C [324, 326]     439.2183+     567     310.1709 (y2); 147.1122 (y1); 199.1438 (b2); 296.1754 (b3); 493.2428 (b7); 111.527 (b6); 111.525 (b6); 101.5143 (b1); 151.521 (b6); 348.5489 (b1); 101.648.678 (b1); 101.61.8078 (b1)       HC22     KEYK C [324, 326]     439.2183+     567     310.1709 (y2); 147.1132 (y1)       HC23     KGK V [327, 328]     307.1427+     2.00     348.1872 (y2); 281.1132 (y1)     101.1132 (y1); 161.369 (y1); 167.1073 (b2); 301.1498 (b3)       HC24     KVNK A [329,  | HC17         | R.TPEVT <u>C</u> VVVDVSHEDPEVK.F [262, 280]   | 713.6810+++   | 17.27                 | 1451.7383 (y13); 1352.6719 (y12); 1253.6011 (y11); 1154.5336 (y10); 1039.5043 (y9); 940.4366 (y8);<br>853.4050 (y7); 716.3464 (y6); 587.3035 (y5); 472.2759 (y4); 375.2241 (y3); 246.1809 (y2); 147.1123<br>(y1); 102.0548 (b1); 199.1074 (b2); 328.1496 (b3); 427.2175 (b4); 528.2655 (b5); 688.3004 (b6);<br>886.4346 (b8); 985.5012 (b9); 1100.5266 (b10); 1199.6048 (b11) ); 1286.6326 (b12)  |
| HC19   K.TKPR.E [295, 298]   501.3149+   2.40   175.1183 (t); 230.1512 (b2).     HC20   R.EEQVNSTYR.V [299, 307]   595.2588++   10.20   803.3704 (bb): 560.2662 (y4): 439.2217 (y3).     HC21   R.VVSVLTVLHQDWLNGK.E [308, 323]   904.5058++   10.20   803.3704 (b2): 460.3070 (y5): 526.2568 (y4): 439.2217 (y3).     HC22   K.EVK.C [324, 326]   439.2183+   567   310.1709 (y2): 147.1122 (y1): 199.1439 (b2): 286.1754 (b1); 119.6743 (b1); 1377.7482 (b12); 147.1132 (y1).     HC22   K.EVK.C [324, 326]   439.2183+   567   310.1709 (y2): 147.1132 (y1).   160.8843 (b1); 119.6743 (b1); 119.6743 (b1); 119.6743 (b1); 119.6743 (b1); 110.6863 (b1).     HC22   K.EVK.C [327, 328]   307.1427+   2.30   147.1122 (y1): 161.3089 (b1)     HC24   K.VSINK.A [329, 332]   447.2567+   2.30   348.1872 (s); 281.1552 (y2): 147.1130 (y1): 187.1073 (b2); 301.1498 (b3).     HC24   K.VSINK.A [341, 344]   448.2760+   5.60   234.1439 (y2): 147.1137 (y1): 215.1399 (b2); 302.173 (b3).     HC25   K.ALPAPIEK.T [333, 340]   419.7507++   13.07   767.4689 (y7): 655.3342 (b1); 602.2707 (b5): 563.3545 (b6); 692.3977 (b7).     HC26   K.GPV.PSLAS.1341, 3441   448.2760+   5.60   234.1439 (y2): 147.1137 (y1): 215.1399 (b2); 302.173 (b3).   | HC18         | K.FNWYVDGVEVHNAK.T [281, 294]   | 839.3967++    | 17.67                 | 1416.6915 (y12); 1230.6108 (y11); 1067.5472 (y10); 968.4800 (y9); 853.4513 (y8); 796.4317 (y7);<br>697.3622 (y6); 568.3196 (y5); 469.2512 (y4); 332.1928 (y3); 218.1494 (y2); 147.1122 (y1); 262.1182<br>(b2); 448.1976 (b3); 611.2613 (b4); 710.3315 (b5); 825.3561 (b6); 882.3832 (b7); 981.4466 (b8);<br>1110.4916 (b9); 1209.5591 (b10); 1346.6165 (b11); 1460.6575 (b12); 1531.7027 (b13)  |
| Incertist H.V. [239, 301]     1952.59674     1020     903.5104 (ty), 1953.2062 (ty), 133.2075 (ty), 195       HC21     R.VVSVLTVLHQDWLNGK.E [308, 323]     904.50584+     1609.6697 (t/14); 1522.8416 (t/13); 1423.26769 (t/2); 1310.6867 (t/11); 1209.6407 (y/10); 1110.5688 (ty); 997.4862 (ty); 199.1436 (ty); 1313.0686 (ty); 131.206 (ty   | HC19         | K.TKPR.E [295, 298]   | 501.3149+     | 2.40                  | 175.1183 (y1); 230.1512 (b2)<br>803 3704 (v6): 640 3070 (v6): 526 2662 (v4): 430 2217 (v2)  |
| HC22     K.EYK.C [324, 326]     439.2183+     5.67     310.1709 (y2); 147.1132 (y1)       HC23     K.CK.V [327, 328]     307.1427+     2.03     147.1122 (y1); 161.0569 (b1)       HC24     K.VSNK.A [329, 332]     447.2567+     2.30     348.1872 (y3); 261.1552 (y2); 147.1130 (y1); 187.1073 (b2); 301.1498 (b3)       HC25     K.ALPAPIEK.T [333, 340]     419.7507++     13.07     767.4689 (y7); 654.3830 (y6); 557.3364 (y5); 466.2925 (y4); 389.2399 (y3); 276.1553 (y2); 147.1128       HC26     K.TISK.A [341, 344]     448.2760+     5.60     234.1439 (y2); 147.1137 (y1); 215.1399 (b2); 302.1733 (b3)       HC27     K.AK.G [345, 346]     218.1499+     2.03     147.1129 (y1)       HC28     K.GQPR.E [347, 350]     457.2515+     3.43     272.1720 (y2); 175.1181 (y1); 186.0868 (b2)       HC39     R.EPQVYTLPPSR.E [351, 361]     643.8400++     14.33     (b3)     248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC31     K.NQVSLTCLVK.G [367, 376]     581.3180++     16.7     379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     645.3116++++     20.43     1693.7510 (y14); 1594.6710 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10  | HC20         | R.VVSVLTVLHQDWLNGK.E [308, 323]   | 904.5058++    | 22.96                 | 1603.687 (v14); 1522.8416 (v13); 1423.7689 (v12); 1310.6867 (v11); 1209.6407 (v10); 1110.5688 (v9); 997.4862 (v8); 860.4268 (v7); 732.3675 (v6); 617.3403 (v5); 431.2606 (v4); 318.1767 (v3); 204.1340 (v2); 147.1122 (v1); 199.1439 (b2); 286.1754 (b3); 385.2453 (b4); 498.3291 (b5); 599.3741 (b6); 698.4438 (b7); 811.5251 (b8); 948.5889 (b9); 1076.6439 (b10); 1191.6743 (b11); 1377.7482 (b12); 1604.8843 (b14); 1661.8978 (b15)   |
| HC23   K_CK_V [327, 328]   307.1427+   2.03   147.1122 (y1); 161.0369 (b1)     HC24   K.VSNK.A [329, 332]   447.2567+   2.30   348.1872 (y3); 261.1552 (y2); 147.1130 (y1); 187.1073 (b2); 301.1498 (b3)     HC25   K.ALPAPIEK.T [333, 340]   419.7507++   13.07   (767.4689 (y7); 654.3830 (y6); 557.3364 (y5); 486.2925 (y4); 389.2399 (y3); 276.1553 (y2); 147.1128     HC26   K.TISK.A [341, 344]   448.2760+   5.60   234.1439 (y2); 147.1137 (y1); 215.1399 (b2); 302.1733 (b3)     HC27   K.AK G [345, 346]   218.1499+   2.03   147.1129 (y1)     HC28   K.GQPR.E [347, 350]   457.2515+   3.43   272.1720 (y2); 175.1181 (y1); 186.0686 (b2)     HC29   R.EPQVYTLPPSR.E [351, 361]   643.8400++   14.33   83.4492 (y7); 670.3902 (y6); 569.3416 (y6); 456.2560 (y4); 359.2071 (y3); 175.1182 (y1); 355.1563     HC30   R.EEMTK.N [362, 366]   637.2858+   6.67   379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)     HC31   K.NQVSLTGLVK.G [367, 376]   581.3180++   16.73   243.1190 (b1); 342.1818 (b3)     HC32   K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]   643.7148+++   16.73   243.190 (b1); 346.56.309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797 (y9); 948.4813 (y8); 892.4461 (y7); 764.3576 (y6); 667.2997 (y5); 538.2   | HC22         | K.EYK.C [324, 326]  | 439.2183+     | 5.67                  | 310.1709 (y2); 147.1132 (y1)  |
| H122T   H47.2507T   2.30   391.072 (y2), 201.152 (y2), 147.1130 (y1), 167.1073 (b2), 301.1736 (b2), 392.399 (39), 393.276.1553 (y2); 147.1128     HC25   K.ALPAPIEK.T [333, 340]   419.7507++   13.07   767.4689 (y7); 654.3364 (b5); 466.2925 (y4); 399.2399 (39); 276.1553 (y2); 147.1128     HC26   K.TISK.A [341, 344]   448.2760+   5.60   234.1439 (y2); 147.1137 (y1); 215.1399 (b2); 302.1733 (b3)     HC27   K.K.G [345, 346]   218.1499+   2.03   147.1129 (y1)     HC28   K.GQPR.E [347, 350]   457.2515+   3.43   272.1720 (y2); 175.1181 (y1); 186.0868 (b2)     HC29   R.EPQVYTLPPSR.E [351, 361]   643.8400++   14.33   833.4492 (y7); 670.3902 (y6); 569.3416 (y5); 456.2560 (y4); 359.2071 (y3); 175.1182 (y1); 355.1563     HC30   R.EEMTK.N [362, 366]   637.2858+   6.67   379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)     HC31   K.NQVSLTCLVK.G [367, 376]   581.3180++   16.73   243.1090 (b1); 342.1818 (b3)     HC32   K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]   848.7148+++   20.10   310.1755 (y2); 147.1119 (y1); 205.0973 (b2); 388.21611 (b3); 465.210 (b1); 109.3547 (b7); 681.3947 (b8); 892.4461 (y7); 764.3576 (y6); 667.2907 (y5); 538.2611 (y4); 492.211 (b5); 780.3547 (b7); 851.3947 (b8); 892.4416 (y7); 793.302 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6); 778.55 (b14) <t< td=""><td>HC23</td><td>K.<u>C</u>K.V [327, 328]</td><td>307.1427+</td><td>2.03</td><td>147.1122 (y1); 161.0369 (b1)<br/>348 1872 (y2): 261 1552 (y2): 147 1130 (y4): 187 1073 (b2): 304 1409 (b2)</td></t<>  | HC23         | K. <u>C</u> K.V [327, 328]  | 307.1427+     | 2.03                  | 147.1122 (y1); 161.0369 (b1)<br>348 1872 (y2): 261 1552 (y2): 147 1130 (y4): 187 1073 (b2): 304 1409 (b2)   |
| HC20     INSTRUMENT (100)     HOSD (1)     HOSD (1)     (1): 185.1283 (b2): 282.1814 (b3); 353.2192 (b4); 450.2706 (b5); 563.3545 (b6); 692.3977 (b7)       HC26     K.TISK.A [341, 344]     448.2760+     5.60     234.1439 (y2); 147.1137 (y1); 215.1399 (b2); 302.1733 (b3)       HC27     K.K.G [345, 346]     218.1499+     2.03     147.1129 (y1)       HC28     K.GQPR.E [347, 350]     457.2515+     3.43     272.1720 (y2); 175.1181 (y1); 186.0868 (b2)       HC29     R.EPQVYTLPPSR.E [351, 361]     643.8400++     14.33     (b3)     248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC30     R.EEMTK.N [362, 366]     637.2858+     6.67     379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC31     K.NQVSLTCLVK.G [367, 376]     581.3180++     16.73     243.1090 (b1); 342.1818 (b3)       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     20.10     1693.7510 (r14); 1594.6710 (r13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     20.10     1063.5161 (y6); 949.4381 (y6); 892.4161 (b1); 1205.5870 (b11); 1394.6275 (b12); 1595.6536 (b13); 1780.7855 (b14)       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]   | HC25         | K AL PAPIEK T [333-340]   | 419 7507++    | 13.07                 | 767.4689 (y7); 654.3830 (y6); 557.3364 (y5); 486.2925 (y4); 389.2399 (y3); 276.1553 (y2); 147.1128  |
| HC27   KAK.G [345, 346]   218.1499+   2.03   147.1129 (y1)     HC28   K.GQPR.E [347, 350]   457.2515+   3.43   272.1720 (y2); 175.1181 (y1); 186.0868 (b2)     HC29   R.EPQVYTLPPSR.E [351, 361]   643.8400++   14.33   833.4492 (y7); 670.3902 (y6); 569.3416 (y5); 456.2560 (y4); 359.2071 (y3); 175.1182 (y1); 355.1563 (b3)     HC30   R.EEMTK.N [362, 366]   637.2858+   6.67   379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)     HC31   K.NQVSLTQLVK.G [367, 376]   581.3180++   16.73   243.1090 (b1); 342.1818 (b3)     HC32   K.GFYPSDIAVEWESNGQPENNYK.T [377, 388]   581.3180++   167.3   243.1090 (b1); 342.1818 (b3)     HC32   K.GFYPSDIAVEWESNGQPENNYK.T [377, 388]   648.7148+++   20.1755 (y2); 147.1119 (y1); 205.0973 (b2); 368.1611 (b3); 465.2116 (b4); 667.221 (b5); 780.3547 (b7); 851.3947 (b8); 904.4615 (b9); 1079.5090 (b10); 1265.5870 (b11); 1394.6275 (b12); 1595.6536 (b13); 1780.7855 (b14)     HC33   K.TTPPVLDSDGSFFLYSK.L [399, 415]   625.3116+++   20.93   (v3); 234.1449 (y2); 147.1128 (y1); 203.0932 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6); 724.3839 (b7); 938.4703 (b10); 1070.5037 (b11); 1217.5710 (b12)     HC34   K.LTVDK.S [416, 420]   575.3400+   9.03   262.1395 (y2); 147.1128 (y1); 231.032 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6); 724.3839 (b7); 938.4703 (b10);   | HC26         | K TISK & [3/1 - 2/4]  | 448 2760      | 5.60                  | (y1); 185.1283 (b2); 282.1814 (b3); 353.2192 (b4); 450.2706 (b5); 563.3545 (b6); 692.3977 (b7)  |
| HC28     K.GQPR.E [347, 350]     457.2515+     3.43     272.1720 (y2); 175.1181 (y1); 186.0868 (b2)       HC29     R.EPQVYTLPPSR.E [351, 361]     643.8400++     14.33     833.4492 (y7); 670.3902 (y6); 569.3416 (y5); 456.2560 (y4); 359.2071 (y3); 175.1182 (y1); 355.1563       HC30     R.EEMTK.N [362, 366]     637.2858+     6.67     379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC31     K.NQVSLTCLVK.G [367, 376]     581.3180++     16.73     243.1090 (b1); 342.1818 (b3)       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     20.10     1693.7510 (y14); 1594.6710 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797 (y9); 949.4381 (y8); 892.4161 (y7); 764.3576 (y6); 667.2997 (y5); 538.2611 (y4); 424.2201 (y3);       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     20.10     1693.7510 (y14); 1594.6710 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797 (y7); 804.302 (b2); 368.1611 (b3); 465.2116 (b4); 667.221 (b5); 780.3547 (b7); 804.302 (b1); 1079.5090 (b10); 1265.5870 (b11); 1394.6275 (b12); 1595.6536 (b13); 1780.7855 (b14)       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     20.93     1063.5161 (y9); 948.4813 (y8); 891.4625 (y7); 804.4308 (y6); 657.3602 (y5); 510.2934 (y4); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6); 724.3839 (b7); 938.4703 (b10); 1070.5037 (b11); 1217.5710 (b12)       HC33     K.TTPPVL  | HC27         | K.AK.G [345, 346]   | 218.1499+     | 2.03                  | 147.1129 (y1)   |
| HC29     R.EPQVYTLPPSR.E [351, 361]     643.8400++     14.33     833.4492 (y7); 670.3902 (y6); 569.3416 (y5); 456.2560 (y4); 359.2071 (y3); 175.1182 (y1); 355.1563 (b3)       HC30     R.EEMTK.N [362, 366]     637.2858+     6.67     379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC31     K.NQVSLTCLVK.G [367, 376]     581.3180++     16.67     243.1090 (b1); 342.1818 (b3)       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     20.10     1693.7510 (y14); 1594.6710 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797 (y9); 949.4381 (y8); 892.4161 (y7); 764.3576 (y6); 667.2997 (y5); 538.2611 (y4); 424.2021 (y3);       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     20.10     1693.7510 (y14); 1594.6730 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797 (y7); 939.476 (b3); 950.4615 (b9); 607.3907 (b2); 588.2011 (y4); 424.2021 (y3);       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     20.10     1063.5161 (y9); 948.4813 (y6); 891.4625 (y7); 804.4308 (y6); 657.3602 (y5); 510.2934 (y4); 397.2081 (y3); 234.1449 (y2); 147.1125 (y1); 203.1032 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6);       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     20.93     175.1183 (y1)       HC34     K.LTVDK.S [416, 420]     575.3400+     9.03     262.1395 (y2); 147.1126 (y1); 2   | HC28         | K.GQPR.E [347, 350]   | 457.2515+     | 3.43                  | 272.1720 (y2); 175.1181 (y1); 186.0868 (b2)   |
| HC30     R.EEMTK.N [362, 366]     637.2858+     6.67     379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)       HC31     K.NQVSLTCLVK.G [367, 376]     581.3180++     16.73     243.1090 (b1); 342.1818 (b3)       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     16.73     243.1090 (b1); 342.1818 (b3)       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377, 398]     848.7148+++     20.10     1693.7510 (y14); 1594.6710 (y73; 764.3576 (y6); 667.2997 (y5); 538.2611 (y4); 424.2201 (y3); (y6); 949.4381 (y8); 892.4161 (y7); 764.3576 (y6); 667.2997 (y5); 538.2611 (b4); 424.2201 (y3); 310.1755 (y2); 147.1119 (y1); 205.0973 (b2); 368.1611 (b3); 465.2116 (b4); 667.221 (b5); 780.3547 (b7); 803.5161 (y9); 948.4813 (y8); 891.4625 (y7); 804.4308 (y6); 657.3602 (y5); 510.2934 (y4); 397.2081 (b1); 13780.7855 (b14)       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     20.93     1063.5161 (y9); 948.4813 (y8); 891.4625 (y7); 804.4308 (y6); 657.3602 (y5); 510.2934 (y4); 397.2081 (y3); 234.1449 (y2); 147.1125 (y1); 203.1032 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6);       HC33     K.LTVDK.S [416, 420]     575.3400+     9.03     262.1395 (y2); 147.1128 (y1); 215.1385 (b2)       HC35     K.SR.W [421, 422]     262.1506+     2.03     175.1183 (y1)       HC36     R.WQQGNVFSCSVMHEALHNHYTQK.S     934.4266+++     17.13     376.2277 (y3); 147.1130 (y1);   | HC29         | R.EPQVYTLPPSR.E [351, 361]  | 643.8400++    | 14.33                 | 833.4492 (y7); 670.3902 (y6); 569.3416 (y5); 456.2560 (y4); 359.2071 (y3); 175.1182 (y1); 355.1563 (b3)   |
| HC31     K.NQVSLTCLVK.G [367, 376]     581.3180++     16.73     243.1090 (b1); 342.1818 (b3)       HC32     K.GFYPSDIAVEWESNGQPENNYK.T [377,<br>398]     k.GFYPSDIAVEWESNGQPENNYK.T [377,<br>398]     k.87148+++     1693.7510 (y14); 1594.6710 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797<br>(y9); 949.4381 (y8); 892.4161 (y7); 764.3576 (y6); 667.2997 (y5); 538.2611 (y4); 424.2201 (y3);<br>310.1755 (y2); 147.1119 (y1); 205.0973 (b2); 368.1611 (b3); 465.2116 (b4); 667.221 (b5); 780.3547<br>(b7); 851.3947 (b8); 950.4615 (b9); 1079.5090 (b10); 1265.5870 (b11); 1394.6275 (b12); 1595.6536<br>(b13); 1780.7855 (b14)       HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     20.93<br>(y3): 234.1449 (y2); 147.1125 (y1); 203.1032 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6);<br>724.3839 (b7); 938.4703 (b10); 1070.5037 (b11); 1217.5710 (b12)       HC34     K.LTVDK.S [416, 420]     575.3400+     9.03<br>9.02 262.1395 (y2); 147.1128 (y1); 215.1385 (b2)       HC35     K.SR.W [421, 422]     262.1506+     2.03<br>175.1183 (y1)       HC36     R.WQQGNVFSCSVMEALHNHYTQK.S<br>[423, 445]     934.4266+++     17.13<br>136.2277 (y3); 147.1130 (y1); 315.1444 (b2)       HC37     K.SLSLSPG [446, 452] (C-terminal)     660.3680+     13.80     260.1249 (y3); 173.0908 (y2); 201.1224 (b2); 288.1556 (b3); 488.2747 (b5)   | HC30         | R.EEMTK.N [362, 366]  | 637.2858+     | 6.67                  | 379.2018 (y3); 248.1603 (y2); 147.1121 (y1); 259.0912 (b2); 390.1361 (b3)   |
| HC33     K.TTPPVLDSDGSFFLYSK.L [399, 415]     625.3116+++     1063.5161 (y9); 948.4813 (y8); 891.4625 (y7); 804.4308 (y6); 657.3602 (y5); 510.2934 (y4); 397.2081 (y3); 234.1449 (y2); 147.1125 (y1); 203.1032 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6); 724.3839 (b7); 938.4703 (b10); 1070.5037 (b11); 1217.5710 (b12)       HC34     K.LTVDK.S [416, 420]     575.3400+     9.03     262.1395 (y2); 147.1128 (y1); 215.1385 (b2)       HC35     K.SR.W [421, 422]     262.1506+     2.03     175.1183 (y1)       HC36     R.WQQGNVFSQ_SVMHEALHNHYTQK.S<br>[423, 445]     934.4266+++     17.13     376.2277 (y3); 147.1130 (y1); 315.1444 (b2)       HC37     K.SLSLSPG-[446, 452] (C-terminal)     660.3680+     13.80     260.1249 (y3); 173.0908 (y2); 201.1224 (b2); 288.1556 (b3); 488.2747 (b5)   | HC31<br>HC32 | K.GFYPSDIAVEWESNGQPENNYK.T [377,<br>398]  | 848.7148+++   | <u>16.73</u><br>20.10 | 243.1090 (b1); 342.1818 (b3)<br>1693.7510 (y14); 1594.6710 (y13); 1465.6309 (y12); 1279.5533 (y11); 1150.5127 (y10); 1063.4797<br>(y9); 949.4381 (y8); 892.4161 (y7); 764.3576 (y6); 667.2997 (y5); 538.2611 (y4); 424.2201 (y3);<br>310.1755 (y2); 147.1119 (y1); 205.0973 (b2); 368.1611 (b3); 465.2116 (b4); 667.221 (b5); 780.3547<br>(b7); 851.3947 (b8); 950.4615 (b9); 1079.5090 (b10); 1265.5870 (b11); 1394.6275 (b12); 1595.6536<br>(b13); 1780.7855 (b14)  |
| HC34     K.LTVDK.S [416, 420]     575.3400+     9.03     262.1395 (y2); 147.1128 (y1); 215.1385 (b2)       HC35     K.SR.W [421, 42]     262.1506+     2.03     175.1183 (y1)       HC36     R.WQQGNVFSCSVMEALHNHYTQK.S<br>[423, 445]     934.4266+++     17.13     376.2277 (y3); 147.1130 (y1); 315.1444 (b2)       HC37     K.SLSLSPG [446, 452] (C-terminal)     660.3680+     13.80     260.1249 (y3); 173.0908 (y2); 201.1224 (b2); 288.1556 (b3); 488.2747 (b5)  | HC33         | K.TTPPVLDSDGSFFLYSK.L [399, 415]  | 625.3116+++   | 20.93                 | 1063.5161 (y9); 948.4813 (y8); 891.4625 (y7); 804.4308 (y6); 657.3602 (y5); 510.2934 (y4); 397.2081 (y3); 234.1449 (y2); 147.1125 (y1); 203.1032 (b2); 397.2081 (b4); 496.2781 (b5); 609.3581 (b6); 724.3839 (b7); 938.4703 (b10); 1070.5037 (b11); 1217.5710 (b12)   |
| HC36     R.WQQGNVFSC_SVMHEALHNHYTQK.S     934.4266+++     17.13     376.2277 (y3); 147.1130 (y1); 315.1444 (b2)       HC37     K.SLSLSPG-[446, 452] (C-terminal)     660.3680+     13.80     260.1249 (y3); 173.0908 (y2); 201.1224 (b2); 288.1556 (b3); 488.2747 (b5)  | HC34         | K.LTVDK.S [416, 420]  | 575.3400+     | 9.03                  | 262.1395 (y2); 147.1128 (y1); 215.1385 (b2)   |
| Ideal     Idea     Ideal     Ideal <thi< td=""><td>HC36</td><td>R.WQQGNVFS<u>C</u>SVMHEALHNHYTQK.S</td><td>934,4266+++</td><td>2.03</td><td>376.2277 (y3); 147.1130 (y1); 315.1444 (b2)</td></thi<>   | HC36         | R.WQQGNVFS <u>C</u> SVMHEALHNHYTQK.S  | 934,4266+++   | 2.03                  | 376.2277 (y3); 147.1130 (y1); 315.1444 (b2)   |
|   | HC37         | [423, 445]<br>K.SLSLSPG [446, 452] (C-terminal)   | 660,3680+     | 13.80                 | 260.1249 (y3); 173.0908 (y2); 201.1224 (b2); 288.1556 (b3): 488.2747 (b5)   |

#### Table 4. De novo peptide sequencing of bevacizumab biosimilar light chain by MS/MS DIA approach on LCMS-9030

| Peak | Pentide [AA numbers]  | MS            | RT    | MS/MS  |
|------|---|---------------|-------|--|
| No.  | · opine [/ s · institution]   | (m/z&z, mea.) | (min) | (m/z, mea.) matched with predicted transitions in Skyline s/w  |
| LC01 | DIQMTQSPSSLSASVGDR.V [1, 18]  | 939.9467++    | 15.13 | 1391.6845 (y14); 1162.5622 (y12); 1075.5380 (y11); 978.4829 (y10); 691.3380 (y7); 604.2981 (y6);<br>533.2642 (y5); 446.2338 (y4); 347.1694 (y3); 175.1192 (y1); 357.1749 (b3); 1075.5380 (b10)   |
| LC02 | R.VTIT <u>C</u> SASQDISNYLNWYQQKPGK.A<br>[19, 42]                     | 934.4571+++   | 20.83 | 557.3413 (y5); 301.1873 (y3) ; 201.1217 (b2); 948.4503 (b9)  |
| LC03 | K.APK.V [43, 45]  | 315.2036+     | 2.30  | 244.1651 (y2); 147.1123 (y1); 169.0970 (b2)  |
| LC04 | K.VLIYFTSSLHSGVPSR.F [46, 61]   | 881.9708++    | 18.83 | 1437.7128 (y13); 1274.6524 (y12); 1127.5804 (y11); 1026.5336 (y10); 939.5010 (y9); 852.4689 (y8);<br>739.3851 (y7); 602.3267 (y6); 515.2926 (y5); 458.2716 (y4); 359.2037 (y3); 262.1487 (y2); 175.1187<br>(y1); 213.1594 (b2); 326.2435 (b3); 489.3059 (b4); 636.3748 (b5); 737.4263 (b6); 911.4881 (b8);<br>1024.5651 (b9); 1161.6352 (b10); 1248.6674 (b11); 1305.6857 (b12); 1404.7547 (b13)   |
| LC05 | R.FSGSGSGTDFTLTISSLQPEDFATYY <u>C</u> Q<br>QYSTVPWTFGQGTK.V [62, 103] | 1554.0430+++  | 25.23 | 2214.0026 (y18); 2050.9346 (y17); 1887.8758 (y16); 1727.8412 (y15); 1599.7832 (y14); 1471.7274 (y12); 1308.6560 (y12); 1221.6252 (y11); 1120.5782 (y10); 1021.5104 (y9); 924.4617 (y8); 738.3787 (y7); 637.3314 (y6); 490.2612 (y5); 433.2402 (y4); 305.1811 (y3); 248.1590 (y2); 147.1137 (y1); 436.1843 (b5); 523.2132 (b6); 681.2908 (b8); 796.3093 (b9); 943.3770 (b10); 1044.4258 (b11); 1157.5080 (b12); 1259.5585 (b13); 1371.6421 (b14); 1458.6767 (b15); 1545.7069 (b16); 1658.7929 (b17); 1786.8490 (b18); 2346.0809 (b23)                           |
| LC06 | K.VEIK.R [104, 107]   | 488.3092+     | 8.77  | 389.2385 (y3); 260.1968 (y2); 147.1125 (y1); 229.1181 (b2); 342.2025 (b3)  |
| LC18 | K.VEIKR.T [104, 108] (missed 1)                                       | 644.4094+     | 8.47  | 545.3455 (y4); 416.2978 (y3); 303.2140 (y2); 175.1187 (y1); 229.1182 (b2); 342.2010 (b3); 470.3028 (b4)  |
| LC19 | K.RTVAAPSVFIFPPSDEQLK.S [108, 126]<br>(missed 1)                      | 1051.5680++   | 20.50 | 913.4683 (y8)  |
| LC07 | R.TVAAPSVFIFPPSDEQLK.S [109, 126]                                     | 973.5168++    | 21.70 | 1745.9188 (y16); 1674.8718 (y15); 1603.8408 (y14); 1506.7970 (y13); 1419.7409 (y12); 1320.6803 (y11); 1173.6172 (y10); 1060.5208 (y9); 913.4617 (y8); 816.4173 (y7); 632.3204 (y5); 517.2991 (y4); 272.1599 (b3); 626.3482 (b7); 1033.5766 (b10)   |
| LC08 | K.SGTASVV <u>C</u> LLNNFYPR.E [127, 142]                              | 899.4515++    | 23.17 | 1481.7463 (y12); 1394.7272 (y11); 1295.6600(y10); 1196.5948 (y9); 1036.5639 (y8); 923.4729 (y7); 810.3884 (y6); 696.3476 (y5); 582.3045 (y4); 435.2353 (y3); 272.1711 (y2); 175.1189 (y1)  |
| LC09 | R.EAK.V [143, 145]  | 347.1935+     | 2.07  | 218.1494 (y2); 147.1124 (y1); 201.0867 (b2)  |
| LC10 | K.VQWK.V [146, 149]   | 560.3175+     | 10.77 | 461.2505 (y3); 333.1920 (y2); 147.1125 (y1); 228.1339 (b2); 414.2133 (b3)  |
| LC11 | K.VDNALQSGNSQESVTEQDSK.D [150,<br>169]                                | 1068.4870++   | 11.20 | 1807.8389 (y17); 1736.7939 (y16); 1495.6525 (y14); 1408.6193 (y13); 1351.5945 (y12); 1237.5567 (y11); 1150.5233 (y10); 1022.4644 (y9); 893.4209 (y8); 806.3889 (y7); 707.3203 (y6); 606.2720 (y5); 477.2291 (y4); 349.1714 (y3); 234.1445 (y2); 147.1119 (y1); 215.1024 (b2); 329.1450 (b3); 400.1817 (b4); 513.2674 (b5); 641.3246 (b6); 728.3561 (b7); 785.3742 (b8); 899.4214 (b9); 986.4566 (b10); 1114.5108 (b11); 1243.5513 (b12); 1330.5941 (b13); 1429.6534 (b14); 1530.7038 (b15); 1659.7397 (b16); 1787.8008 (b17); 1902.8346 (b18); 1989.8593 (b19) |
| LC12 | K.DSTYSLSSTLTLSK.A [170, 183]   | 751.8830++    | 17.17 | 1300.7064 (y12): 1199.6517 (y11): 1036.5889 (y10): 949.5564 (y9): 836.4713 (y8): 749.4399 (y7):<br>662.4086 (y6): 561.3599 (y5): 448.2763 (y4): 347.2284 (y3): 234.1445 (y2): 147.1119 (y1): 203.0654<br>(b2): 304.1140 (b3): 467.1782 (b4): 554.2020 (b5): 667.2939 (b6): 754.3271 (b7): 841.3568 (b8):<br>942.4002 (b9): 1055.4876 (b10): 1156.5379 (b11): 1269.6205 (b12): 1356.6489 (b13)  |
| LC13 | K.ADYEK.H [184, 188]  | 625.2830+     | 7.60  | 439.2176 (y3); 147.1110 (y1); 350.1302 (b3)  |
| LC14 | K.HK.V [189, 190]   | 284.1717+     | 2.00  | 147.1125 (y1); 138.0661 (b1)   |
| LC15 | K.VYACEVTHQGLSSPVTK.S [191, 207]                                      | 938.4586++    | 13.43 | 531.3121 (y5); 444.2809 (y4); 960.4259 (b8)  |
| LC16 | K.SFNR.G [208, 211]   | 523.2572+     | 7.73  | 436.2312 (y3); 289.1615 (y2); 175.1188 (y1); 88.0390 (b1); 235.1074 (b2); 349.1499 (b3)  |
| LC17 | R.GE <u>C</u> [212, 214] (C-terminal)                                 | 365.1131+     | 2.53  | 308.0896 (y2); 179.0484 (y1); 187.0712 (b2)  |

Here we present peptide ALPAPIEK [332, 339] (HC25) as an example to illustrate the DIA workflow for *de novo* peptide sequencing. After trypsin digestion, the peptide was measured by LCMS-9030 (Q-TOF) both in MS and DIA events. Utilizing the MS-DIAL s/w, the MS data of HC25 including m/z (419.7507++) and retention time (13.07 min) and its corresponding MS/MS spectral data (e.g., 147.1128, 185.1283, 276.1553...) were extracted (Refer to the bottom right in **Figure 1**). In Skyline s/w, y and b ion transitions of HC25 were predicted (Refer to the bottom left in **Figure 1**). The extracted MS/MS data was well matched with the predicted transitions, which verify amino acid sequence of peptide ALPAPIEK.

### D. Post-translational modifications (PTMs)

In addition to providing primary structure confirmation of mAb, peptide mapping by Q-TOF can determine PTMs, such as functional group addition (e.g., phosphorylation, acylation, glycosylation, etc.) and change in physico-chemical properties of amino acids (e.g., citrullination, deamidation, oxidation, etc.). We didn't pay too much attention to PTMs in the present report, however, some PTMs, such as N-glycosylation of peptide HC20 with G0F (*m*/*z* 1317.5273++ @ 9.67 min) and c-terminal lysine processing (HC37) were observed.

# Conclusions

A simple and straightforward MS/MS DIA approach on Shimadzu LCMS-9030 (Q-TOF) mass spectrometer was demonstrated for in-depth peptide mapping of mAb based on *de novo* peptide sequencing.

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