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on behalf of

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PO#:

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**Acknowledgements**

On behalf of Bioinformatics Solutions Inc., we would like to take this opportunity to thank you for choosing PEAKS AB Service for your antibody sequencing needs. It is a pleasure to be working with you.

With this, we are delighted to present to you the successfully decoded protein sequences of the heavy and light chains of your target antibody: {{ Sample\_Name }}.

If you have any questions about our service, please feel free to contact us.

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1. **Antibody Sample Description**

|  |  |
| --- | --- |
| Sample Name: | {{ Sample\_Name }} |
| Antibody Type: |  |
| Species Type: |  |
| Natural or Artificial: |  |
| Quantity: | 200ug lyophilized from 0.22um filtered PBS |
| Reference Sequence Available? | N/A |

1. **Abbreviations**

|  |  |
| --- | --- |
| CDR | Complementarity-Determining Region |
| Da | Dalton |
| DTT | Dithiothreitol |
| ETD | Electron Transfer Dissociation |
| EThcD | Electron-Transfer/Higher-energy Collision Dissociation |
| FR | Framework Region |
| HCD | Higher-energy Collisional Dissociation |
| HPLC | High-Performance Liquid Chromatography |
| LC-MS/MS | Liquid Chromatography - Tandem Mass Spectrometry |
| mAb | Monoclonal Antibody |
| MS | Mass Spectrometry |
| PSM | Peptide-Spectrum Match |
| Q-TOF | Quadrupole Time-of-Flight [mass spectrometer] |

1. **Experimental Procedure**

In-solution endoproteinase digestions of the monoclonal antibody (mAb) were performed for mAb sequencing analysis. The antibody was reduced with DTT and alkylated using iodoacetamide. The sample was equally divided into 5 aliquots for 5 individual enzyme digestions: Asp N, Chymotrypsin, Elastase, Trypsin, and Pepsin following manufacturer’s instructions. After reversed phase cleanup of each digest, samples were kept frozen until mass spectrometry analysis as described below.

**Mass Spectrometry**

Intact Mass Measurement

Intact mass analysis with DTT reduction and PNGase F deglycosylation followed by LC-MS analysis were performed on a Thermo Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer, equipped with a heated electrospray ionization source in positive ion mode with a Thermo Fisher Ultimate 3000 RSLCnano HPLC System. The sample was loaded onto a Thermo Fisher MABPAC RP, ANALYTICAL 4µM, 3.0X50MM column, held at 70°C. The protein was eluted at a rate of 500uL/min using a non linear gradient of 10-70% acetonitrile in 0.1% formic acid. MS spectra were acquired by using full scans at 15000 resolution in the orbitrap using high mass range scanning 850-4000m/z. Maximum injection time was limited to 100ms with an AGC target of 100000. Ten micro scans were employed and the RF lens was set to 45%. 15V of in source CID was applied.

LC-MS/MS Analysis

The digests were analyzed on an Orbitrap analyzer (Orbitrap Fusion Lumos, Thermo Fisher Scientific). Both full MS scans and MS2 scans were acquired in the high resolution Orbitrap mass analyzer. MS2 data were acquired using HCD and ETD followed by HCD (EThcD) fragmentation methods. All raw data files were used for data analysis using the PEAKS AB 2.0 software.

1. **Antibody Heavy Chain**
   1. **Amino Acid Sequence of Heavy Chain**

|  |  |  |
| --- | --- | --- |
| **{%tr for item in hsequence %}** | | |
| {{item.llabel}} | {{ item.col }} | {{item.rlabel}} |
| **{%tr endfor %}** | | |

* 1. **Intact Mass**

**Calculated Average Mass (*Mc*):** {{ heavy\_chain\_cmass }} Da^

^ Truncation of the C-terminal lysine (-128.17 Da) is commonly observed in monoclonal antibody samples.

Protein N-terminal Pyro-Glu from Q (-17.03 Da) is not considered.

**Measured Average Mass with 1\*KLoss, 1\*Pyro-Glu from Q, 1\*A2G0F (*Mm*):** {{ heavy\_chain\_rmass }} Da ‡

**Average Mass Difference:**

*Mm* – *Mc* = {{ heavy\_chain\_rmass }} – 1444.53 – ({{ heavy\_chain\_cmass }} – 128.17 – 17.03) = 0.79 Da ¶

¶ If the absolute mass difference is equal or smaller than 2 Da for heavy chains, it is regarded as an excellent match between the calculated and measured average masses.

* 1. **Peptide Mapping at 1% of FDR at Peptide-Spectrum Match Level**

Amino Acid Confidence: **RED**>95% **BLUE**>85% **BLACK**<85%

**{%p for item in FDR\_hmap %}**

**{{ item.img }}**

**{%p endfor %}**

Note: 11 N-linked glycans were detected.

* 1. **Typical Peptides Selected for Variable Region**

{{ typical\_hpeptide\_image }}

Table 4.1 Abundant peptides selected for heavy chain sequence validation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Position** | **Enzyme** | **#PSMs** | **Precursor**  **Mass** | **Error**  **(PPM)** | **Peptide Abundance** | **Peptide Sequence** |
| **{%tr for item in hpeptides %}** | | | | | | |
| {{ item.position }} | {{ item.enzyme }} | {{ item.psm }} | {{ item.mass }} | {{ item.ppm }} | {{ item.abundance }} | {{ item.sequence }} |
| **{%tr endfor %}** | | | | | | |

**{%p for item in typical\_peptide\_hmap %}**

**{{ item.title }}  
{{ item.img }}**

**{%p endfor %}**

* 1. **Ile/Leu Differentiation**

To differentiate between the isobaric amino acids, isoleucine and leucine, EThcD data, PEAKS AB I/L differentiation algorithm, and manual check are performed.

Table 4.2Ile/Leu differentiation for the assembled sequence

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Region** | **Position** | **Differentiation** | **Confidence** | |
| **{%tr for item in htIL %}** | | | |
| {{ item.region }} | {{ item.position }} | {{ item.differentiation }} | {{ item.confidence }} |
| **{%tr endfor %}** | | | |

1. **Antibody Light Chain**
   1. **Amino Acid Sequence**

|  |  |  |
| --- | --- | --- |
| **{%tr for item in lsequence %}** | | |
| {{item.llabel}} | {{ item.col }} | {{item.rlabel}} |
| **{%tr endfor %}** | | |

* 1. **Intact Mass**

**Calculated Average Mass (*Mc*):** {{ light\_chain\_cmass }} Da

**Measured Average Mass (*Mm*):** {{ light\_chain\_rmass }} Da

**Average Mass Difference:** *Mm* – *Mc* = {{ light\_chain\_rmass }} – {{ light\_chain\_cmass }} = -2.11 Da ¶

¶ If the absolute mass difference is smaller than 2 Da for light chains, it is regarded as an excellent match between the calculated and measured average masses.

* 1. **Peptide Mapping at 1% of FDR at Peptide-Spectrum Match Level**

Amino Acid Confidence: **RED**>95% **BLUE**>85% **BLACK**<85%

**{%p for item in FDR\_lmap %}**

**{{ item.img }}**

**{%p endfor %}**

* 1. **Typical Peptides Selected for Variable Region**

{{ typical\_lpeptide\_image }}

Table 5.1 Abundant peptides selected for light chain sequence validation

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Position** | **Enzyme** | | **#PSMs** | **Precursor**  **Mass** | **Error**  **(PPM)** | | **Peptide Abundance** | **Peptide Sequence** | | |
| **{%tr for item in lpeptides %}** | | | | | | | | | |
| {{ item.position }} | {{ item.enzyme }} | {{ item.psm }} | | {{ item.mass }} | {{ item.ppm }} | {{ item.abundance }} | | | {{ item.sequence }} |
| **{%tr endfor %}** | | | | | | | | | |

**{%p for item in typical\_peptide\_lmap %}**

**{{ item.title }}  
{{ item.img }}**

**{%p endfor %}**

* 1. **Ile/Leu Differentiation**

To differentiate between the isobaric amino acids, isoleucine and leucine, EThcD data, PEAKS AB I/L differentiation algorithm, and manual check are performed.

Table 5.2Ile/Leu differentiation for the assembled sequence

|  |  |  |  |
| --- | --- | --- | --- |
| **Region** | **Position** | **Differentiation** | **Confidence** |
| **{%tr for item in ltIL %}** | | | |
| {{ item.region }} | {{ item.position }} | {{ item.differentiation }} | {{ item.confidence }} |
| **{%tr endfor %}** | | | |