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Final Report

Peptide mapping of Protein X by 1D-nanoLCMS

Report number:	MS-R30000-1
Report date:	20th August 20XX
Project number:	30000
Client name:	Matthew Fitzhenry
Client organisation:	APAF
Client address:	Level 4, 4 Wally's Walk Macquarie University
Client contact number:	(02) 9850 6201
Client email:	info.apaf@mq.edu.au
Date sample(s) received:	10th August 20XX
Number of samples:	1
Project leader:	Matthew Fitzhenry
Authorised by:	Muhammad A. Zenaidee, PhD
APAF email:	info.apaf@mq.edu.au
Attachments (-):	-

The results apply to the sample(s) as received.

As per APAF Terms and Conditions, **samples will be retained for a period of thirty (30) days** and **testing records will be accessible for a period of three (3) years** from the date of reporting results unless other arrangements have been made; refer to Clause 11.1 (sample retention) and Clause 10.3 (test records) for conditions that apply.

Acknowledgment: To comply with our NCRIS operating grant, we require that any publication arising from access to the facility acknowledge the contribution of APAF staff and include the statement "***Aspects of this research have been facilitated by access to the Australian Proteome Analysis Facility supported under the Australian Government's National Collaborative Research Infrastructure Strategy (NCRIS)***".

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SAMPLE DETAILS

- Protein X, 150 mg/mL (APAF Sample code: S0004334)

METHOD DETAILS

- Sample was diluted 1:10 and 1:100 with 100 mM TEAB
- 50 µg of sample was taken for each of the three enzymatic digestions, 20 µg was taken for microwave-assisted acid hydrolysis (MWAH)
- Each enzyme treated sample was reduced with 10 mM DTT at 60°C for 1 hr, the MWAH sample was reduced with 20 mM DTT at 60°C for 20 min
- Enzyme treated samples were alkylated with 20 mM IAA in dark for 20 min, then quenched with DTT
- 50% TFA was added to the MWAH sample. The tube was sealed tightly then treated for 8 min in a 800W microwave oven in a 500 mL water bath
- Enzyme treated samples were individually treated with either 2 µg trypsin, 2 µg chymotrypsin or 5 µg Glu-C overnight at 37°C
- MWAH treated sample was transferred to a new tube then dried completely
- Enzyme treated samples were acidified with TFA then desalted and concentrated with a reverse phase SDB tip. Peptides were eluted with acetonitrile / ammonium hydroxide then dried completely
- All samples were reconstituted with 50 µL of 0.1% formic acid
- 4 µL enzyme treated samples were added to vials containing 16 µL 0.1% formic acid prior to analysis

Data acquisition

Mass spectrometer: QExactive (Thermo)
NanoLC system: Easy nLC-1000
Trap column: Halo-C18, 160Å, 2.7µm, 150 µm x 3.5 cm
Analytical column: Halo-C18, 160Å, 2.7µm, 150 µm x 15 cm
Loading buffer: 99.9% water, 0.1% formic acid
Mobile phase A: 99.9% water, 0.1% formic acid
Mobile phase B: 99.9% acetonitrile, 0.1% formic acid

NanoLC ESI MS/MS data acquisition

Nano-LC method

10 µL of each sample (approx. 2 µg) was injected onto the peptide trap column using 0.1% formic acid. The peptide trap was then switched in-line with the analytical column. Peptides were eluted from the trap into analytical column with a linear gradients of mobile phase B (1-30%) over 90 min with a flow rate of 300 nL/min across the gradient. The eluent from the trap was separated over the analytical column.

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MS method

The column eluent was directed into the ionization source of the mass spectrometer. A 2.3 kV electrospray voltage was applied via a liquid junction upstream of the column. Peptide precursors from 350 to 1850 m/z were scanned at 70k resolution. The 10 most intense ions of the preceding survey scan were fragmented by HCD using normalized collision energy of 27 with an isolation width of 4.0 *m/z*. Only precursors with charge state equal to 2 or more were subjected to MS/MS analysis. The MS method had a minimum signal required value of 1.3×10^4 for MS^2 triggering, an AGC target value of 1×10^6 for MS, 1×10^5 for MS^2 and a maximum injection time of 60 ms for MS^2. MS/MS scan resolution was set at 17.5k. The dynamic exclusion was set to 20 seconds. Raw data was exported into MGF format using the RawConverter 1.1.0.22 program (Scripps Institute, USA).

The MGF was searched with ProteinPilot v.5 (SCIEX) against a database containing the Protein X sequence combined with sequences from E. coli (39,242 sequences), obtained from Uniprot. Additionally, the microwave-assisted acid hydrolysis data was searched using BioPharma Finder v3.0 (Thermo Scientific). The sequence coverage report was generated (GenePattern).

Result

100% sequence coverage of Protein X

Sample name: Protein X
Protein: Protein X
Sequence length: 153

Data used:
Trypsin: 190701_P30700_Protein X_TRYP_QE1_1_PeptideSummary.txt
Chymotrypsin: 190702_P30700_Protein X_CHYM_QE1_1_PeptideSummary.txt
GluC: 190701_P30700_Protein X_GLUC_QE1_1_PeptideSummary.txt
MWAH: 190701_P30700_Protein X_MWAH_QE1_1.txt

>95% confidence shown in green. Overall coverage: 100.0%
>50% confidence shown in yellow. Overall coverage: 100.0%
>0% confidence shown in red. Overall coverage: 100.0%

```
                      10         20         30         40         50         60
Overall       MRPSGRKSSK MQAFRIWDVN QKTFYLRNNQ LVAGYLQGPN VNLEEKIDVV PIEPHALFLG
Sequence      MRPSGRKSSK MQAFRIWDVN QKTFYLRNNQ LVAGYLQGPN VNLEEKIDVV PIEPHALFLG
Trypsin          SGRKSSK MQAFRIWDVN QKTFYLRNNQ LVAGYLQGPN VNLEEKIDVV PIEPHALFLG
Chymotrypsin     SGRKSSK MQAFRIWDVN QKTFYLRNNQ LVAGYLQGPN VNLEEKIDVV PIEPHALFLG
GluC                     MQAFRIWDVN QKTFYLRNNQ LVAGYLQGPN VNLEEKIDVV PIEPHALFLG
MWAH          MRPSGRKSSK MQAFRIWDVN QKTFYLRNNQ LVAGYLQGPN VNLEEKIDVV PIEPHALFLG
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                 70         80         90         100        110        120
Overall     IHGGKMCLSC VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW
Sequence    IHGGKMCLSC VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW
Trypsin     IHGGKMCLSC VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW
Chymotrypsin IHGGKMCLSC VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW
GluC        IHGGKMCLSC VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW
MWAH        IHGGKMCLSC VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW

                130        140        150
Overall     FLCTAMEADQ PVSLTNMPDE GVMVTKFYFQ EDE
Sequence    FLCTAMEADQ PVSLTNMPDE GVMVTKFYFQ EDE
Trypsin     FLCTAMEADQ PVSLTNMPDE GVMVTKFYFQ EDE
Chymotrypsin FLCTAMEADQ PVSLTNMPDE GVMVTKFYFQ EDE
GluC        FLCTAMEADQ PVSLTNMPDE GVMVTKFYFQ EDE
MWAH        FLCTAMEADQ PVSLTNMPDE GVMVTKFYFQ EDE
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Comments

[c] Information was provided by the customer.

OPINIONS AND INTERPRETATIONS

Interpretation and/or detailed discussions may be required to fully understand the results presented to you. APAF is committed to assist our clients/collaborators to maximise the value from their results through these consultations. It should be noted that if these results are to be incorporated into a publication then APAF will be pleased to supply further details/methodology as required by the publishing journal.

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