MACQUARIE University
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APAF Analytical Services Report

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APAF
australian proteome analysis facility

Final Report

Intact Mass Analysis of Protein X and Protein Y

Report number:	MS-R30001-1
Report date:	20th August 20XX
Project number:	30001
Client name:	Matthew Fitzhenry
Client organisation:	APAF
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Client contact number:	(02) 9850 6201
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Date sample(s) received:	10th August 20XX
Number of samples:	2
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)".***

SAMPLE DETAILS

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

METHOD DETAILS

- Each sample was diluted 1:10 with water
- Diluted Protein X and Protein Y further with 1:15 of 2% ACN 0.1% formic acid
- Added 10 µL to vial plus 140 µL 2% ACN 0.1% formic acid

Data acquisition

Mass spectrometer: QExactive Plus (Thermo)
LC system: Agilent 1260 HPLC
Analytical column: XBridge Protein BEH C4 3.5 µm, 300Å, 2.1mm x 150mm (Waters)
Mobile phase A: 99.9% water, 0.1% formic acid
Mobile phase B: 99.9% acetonitrile, 0.1% formic acid

LC intact mass data acquisition

LC method

10 µL of each sample was injected onto the analytical column using 20% mobile phase B separately. Protein was eluted into analytical column with a linear gradients of mobile phase B (20-80%) over 27 min with a flow rate of 200 µL/min across the gradient.

MS method

The column eluent was directed into the ionization source of the mass spectrometer. A 3.5 kV electrospray voltage was applied. Protein components were scanned at 70k resolution from 600 to 3,000 m/z. The MS method had an AGC target value of 1×10^6, 320°C temperature, S-lens 60, Protein Mode, 0.2x pressure.

Results

The intact mass analysis shows Protein X (Figure 1) and Protein Y (Figure 2) without disulfide bonding.

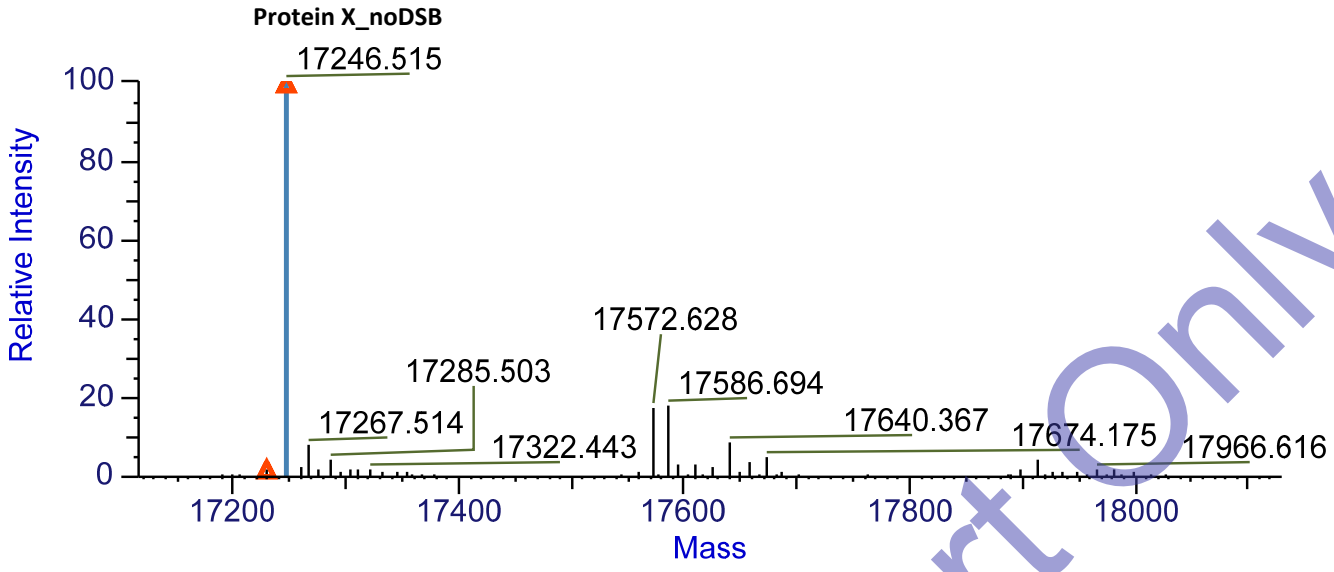

Figure 1: Deconvoluted protein spectrum at retention time = 7 min of Protein X

Protein Name	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Sum Intensity	Relative Abundance
Protein X_noDSB	17246.515	17246.461	3.1	2.75E+06	100.0
	17586.694	0.000	0.0	5.10E+05	18.5
	17572.628	0.000	0.0	4.80E+05	17.4
	17640.367	0.000	0.0	2.42E+05	8.8
	17267.514	0.000	0.0	2.23E+05	8.1
	17674.175	0.000	0.0	1.40E+05	5.1

Table 1: Masses of peaks found above 5% intensity relative to molecular mass peak in Protein X

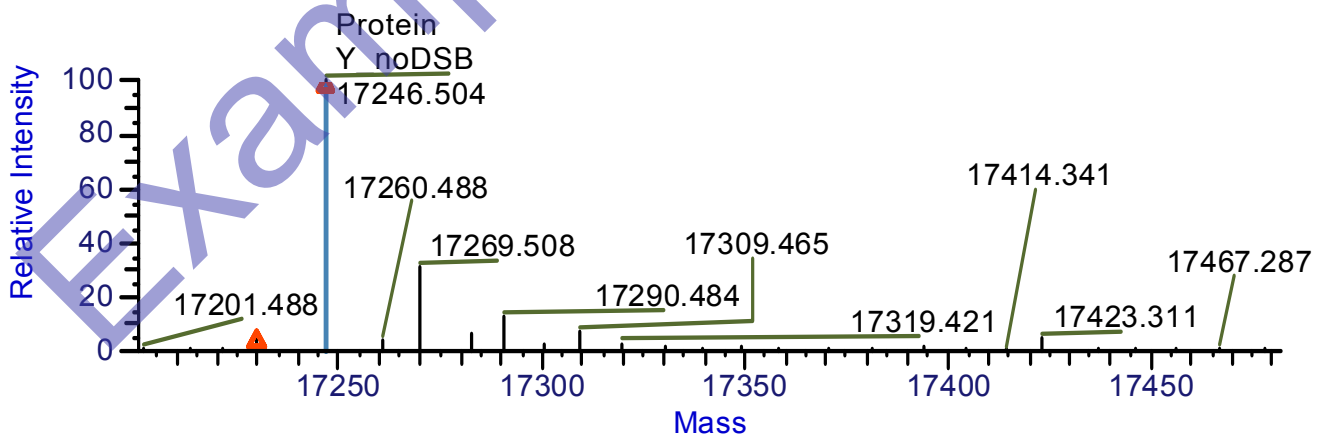

Figure 2: Deconvoluted protein spectrum at retention time = 7 min of Protein Y

Protein Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Sum Intensity	Relative Abundance
Protein Y_noDSB		17246.504	17246.461	2.5	1.50E+08	100.0
		17269.508	0.000	0.0	4.76E+07	31.7
		17290.484	0.000	0.0	2.00E+07	13.3
		17309.465	0.000	0.0	1.17E+07	7.8
		17282.462	0.000	0.0	1.04E+07	7.0
		17423.311	0.000	0.0	7.71E+06	5.2
Protein Y_noDSB_mod	1xGln->Pyro-Glu	17229.486	17229.434	3.0	7.59E+06	5.1

Table 2: Masses of peaks found above 5% intensity relative to molecular mass peak in Protein Y

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.