APAF Analytical Services Report

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Final Report

Protein/Peptide Identification by LC MS/MS Analysis

Report number:	MS-R30001-1
Report date:	11th March 20XX
Project number:	30001
Client name:	Thiri Winn
Client organisation:	APAF
Client address:	Level 4, 4 Wally's Walk Macquarie University, NSW, 2109, AUSTRALIA
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Date sample(s) received:	1st March 20XX
Number of samples:	5
Project leader:	Thiri Winn
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Attachments	Yes (xxx.xlsx)

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

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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 (National Research Infrastructure for Australia) operating grant, we request that any publication arising from access to the facility acknowledge the contribution of APAF staff and include the statement "***This study/project/research used NCRIS-enabled Australian Proteome Analysis Facility (APAF) infrastructure***".

Accreditation Number: 20344
Accredited for compliance with ISO/IEC 17025 - Testing

SAMPLE DETAILS

- Sample 1 (APAF Sample code: S0000001)
- Sample 2 (APAF Sample code: S0000002)
- Sample 3 (APAF Sample code: S0000003)
- Sample 4 (APAF Sample code: S0000004)
- Sample 5 (APAF Sample code: S0000005)

METHOD DETAILS

- SOP used: MS-080_Cell and Tissue lysis and digestion protocol for DDA and DIA MS_V4

In brief:

- Each sample was resuspended in x µL of digestion solution (1% SDC, 100 mM TEAB)
- Protein concentrations were determined
- X µg of each sample was taken for digestion
- Samples were cleaned up by SDB stage tip.
- Cleaned digested samples were dried completely.
- Each sample was resuspended in x µL of loading buffer (0.1 % formic acid)
- X µL (x µg) of each sample was taken for LC MS/MS analysis

DATA ACQUISITION DETAILS

- SOP used: MS-101_HFX Exploris Data Acquisition_V1

Instrument:

Mass spectrometer:	Orbitrap Exploris (Thermo Fisher Scientific)
NanoLC system:	Ultimate 3000 UHPLC System (Thermo Fisher Scientific)
Trap column:	300 µm × 5 mm, C18 PepMap 100, 5µm, 100 Å (Thermo)
Analytical column:	Dr Maisch Reprosil-Pur 120 C18-AQ 1.9µm, 75µm × 30 cm (self-packed)
Loading buffer:	0.1% formic acid
Mobile phase A:	0.1% formic acid
Mobile phase B:	80% acetonitrile, 0.1% formic acid

LC method:

- Sample was injected onto a reverse-phase peptide trap for pre-concentration and desalted with loading buffer.
- Peptides were eluted from the analytical column using a linear gradient of mobile phase B (2.5 - 37.5%) at a flow rate of 300 nL/min over a 60 min period.

MS method (Data Dependent Acquisition):

- The column eluent was directed into the ionization source of the mass spectrometer operating in positive ion mode.

Full Scan		**MS²**	
Scan Range (m/z)	350-1450	Isolation Window (m/z)	1.2
Orbitrap Resolution	60000	Collision Energy Type	Normalized
AGC Target	Standard	HCD Collision Energies (%)	27
Maximum Injection Time (ms)	25	Orbitrap Resolution	15000
Monoisotopic Precursor Selection Filter (MIPS)		AGC Target	Standard
Intensity Threshold	5.00E+03	Maximum Injection Time (ms)	25
Include charge state(s)	2-6	Microscans	1
Exclusion duration (s)	15	Data Type	Centroid
Total duty cycle time (s)	1.5		

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DATA PROCESSING DETAILS

Software used	Proteome Discoverer
Version	2.5
Enzyme Name	Trypsin (Full)
Database	uniprotkb_homo_sapiens_AND_reviewed_tru_2025_01_28.fasta
Max. Missed Cleavage Sites	2
Min. Peptide Length	5
Max. Peptide Length	144
Precursor Mass Tolerance	10 ppm
Fragment Mass Tolerance	0.02 Da
Dynamic Modifications	Oxidation (M)
Static Modifications	Carbamidomethyl (C)
FDR and result display filters	Protein, Peptide, PSM FDR < 1 % (Excel files)

RESULTS

Sample preparation, data acquisition, data analysis, data processing and reporting were performed (XX/XX/XXXX-XX/XX/XXXX):

Proteomic analysis of the samples resulted in identification of xxx proteins in at least one of samples. A list of proteins (xxx.xlsx) is enclosed with the report. The abundances of the matched proteins are shown for the different samples with the abundance column showing the different samples (Column “Abundance: Sample 1”, “Abundance: Sample 2”, “Abundance: Sample 3”, “Abundance: Sample 4”, and “Abundance: Sample 5”).

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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