

The Australian Proteome Analysis Facility

Amino Acid Analysis

APAF provides a comprehensive amino acid analysis service for the determination of amino acid composition and/or total protein content of biological samples as well as the quantitation of particular amino acids as required.

AAA-050	Free amino acids in samples such as culture media or physiological samples such as plasma
AAA-100	High sensitivity amino acid analysis of purified protein using gas phase hydrolysis
AAA-200	Quantitative amino acid analysis of complex samples, such as foodstuffs, using liquid hydrolysis
AAA-SP88	Sample preparation/desalting
AAA-500	Special amino acid quantitation†

†Tryptophan, Cysteine, Hydroxyproline, Taurine, Glutamine, Asparagine, etc.

N-Terminal/Edman Sequencing

APAF provides a high quality Edman sequencing service. In particular, we specialise in high sensitivity protein identifications from gels. We also have expertise in full length sequencing of small proteins, such as toxins and QC analysis of biopharmaceuticals under GLP conditions.

NTS-100	Sequencing set up fee (per sample)
NTS-150	Sequencing per Amino Acid
AAA-SP90	Sample Preparation/ elution from Gel

Liquid Chromatography

APAF has excellent facilities for analytical and preparative HPLC-based protein separations. These include proteome analysis based on multidimensional chromatography (MudPIT) and affinity tag methods (eg. iTRAQ). These LC methods complement the 2D gel electrophoresis approach by improving the coverage of low abundance, low molecular weight, hydrophobic membrane and basic proteins as well as enabling the study of protein complexes. We are also able to purify small quantities of protein.

LC-RP	Reversed Phase HPLC
LC-RPPRP	Reversed Phase Prep
LC-METDEV	HPLC method development
LC-IEX	Cation exchange HPLC
LC-VALN	HPLC method development validation
LC-PEPMAP	Peptide mapping
MS-LC-120	2D LC/MS/MS preparation and data acquisition
MS-LC-120N	2D Nano LC/MS/MS preparation and data acquisition
Disulfide Mapping	



Mass Spectrometry

Mass Spectrometry has become one of the leading tools for protein identification, quantitation and sequencing in recent years, APAF has excellent MS facilities and has increased capabilities allowing us to rapidly analyse large numbers of samples by MALDI-TOF (PMF analysis) and MALDI-TOF/TOF (sequencing and more detailed identification) as well as LC/MS/MS, and 2D-LC/MS/MS. The instruments available provide both MALDI and ESI ion sources, with either single stage (MS), two stage (MS/MS) or quadrupole ion trap mass analysers. In addition, associated capillary/nano flow liquid chromatography equipment ensures we can provide a comprehensive range of services.

APAF can now analyse large numbers of samples and provide high sensitivity detection and identification of peptides derived from purified proteins, proteins isolated from gels or peptides separated by liquid chromatography.

Journal publication requires more rigorous to protein identification than just simply Peptide Mass Fingerprinting (PMF).

MALDI-TOF MALDI-MS/MS ESI MS and ESI MS/MS analysis.

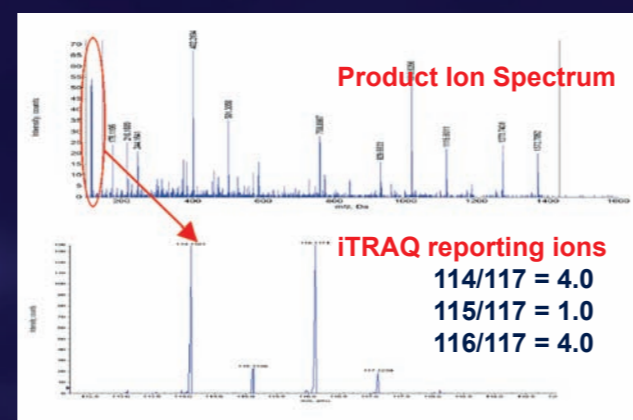
MS-MLD-100	MALDI-TOF PMF sample preparation and data acquisition
MS-MLD-110	MALDI-MS/MS sample preparation and data acquisition
MS-MLD-200	Database search/identification option
MS-MLD-300	MALDI-MS/MS peptide sequencing option
MS-INT	Peptide intact mass analysis
MS-INT-100	Analysis of intact protein by ESI or MALDI
MS-INT-110	Analysis of intact protein by ESI-MS
MS-MAN	Manual MS/MS analysis
MS-MAN-100	Manual ESI MS/MS sample preparation and data acquisition
MS-MAN-200	Database search/identification
MS-MAN-300	Manual MS/MS sequencing De novo sequencing

LC/MS/MS analysis

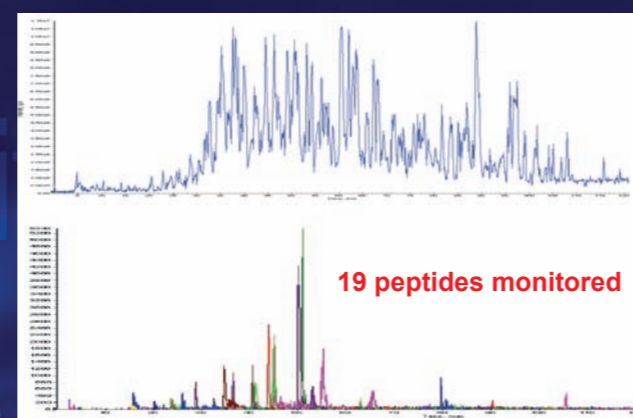
MS-LC-100	LC/MS/MS sample preparation and data acquisition (ESI)
MS-LC-110	LC MALDI LC/MS/MS sample preparation and data acquisition. (MALDI)
MS-LC-120	2D LC/MS/MS sample preparation and data acquisition
MS-LC-200	Database search/identification
MS-LC-300	LC/MS/MS sequencing

Quantitative proteomics by MS/MS

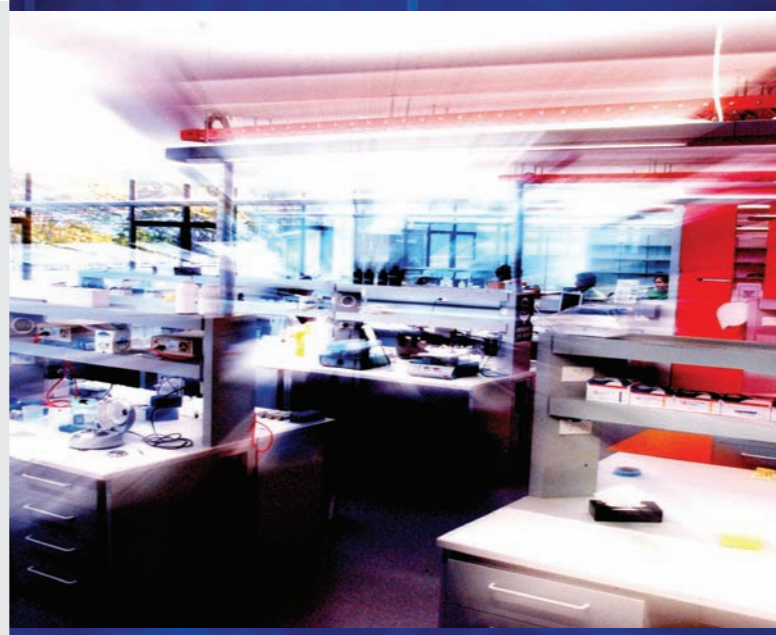
A trend towards quantitative proteomics has led to a number of developments by commercial companies to develop kits in this area. APAF has considerable experience in this area having used iTRAQ™ for a number of years now. This labelling technique enables APAF to quantify and compare the differences in protein expression between samples and controls. The iTRAQ™ work flow includes experimental design, protein extraction, protein reduction and alkylation, trypsin digestion, labelling reagent, strong cation exchange chromatography, reverse phase LC ESI MS/MS data acquisition and data processing. APAF services include all or part of the above procedures depending on your requirements.



Multiple Reaction Monitoring (MRM) is a mass spectrometry technique that is used to identify and quantify targeted molecules in complex samples. It is a highly selective and sensitive technique and as a result pre-fractionation of complex samples is often not required. This assay is a particularly useful technique for screening biomarker candidates in large sample sets. Multiple reactions can be targeted in each mass spectrometry run.



Please visit our website for more information on these protocols. (www.proteome.org.au)



SILAC (stable isotope labeling by amino acids in cell culture)

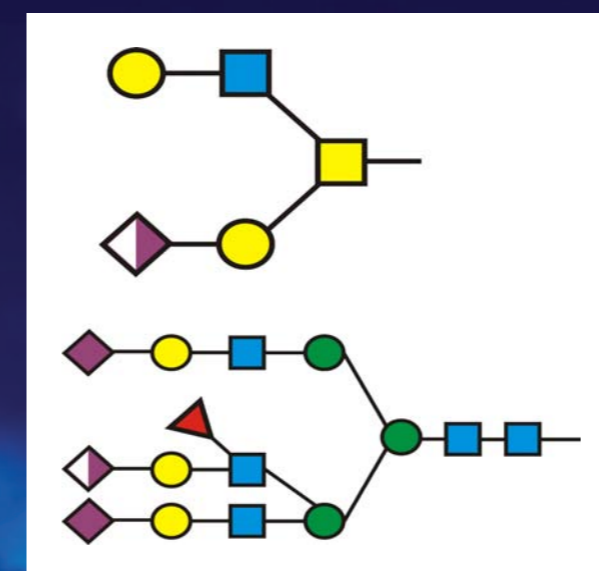
The technique is applicable to all cell culture systems where defined media allows amino acid substitution with "heavy" variants. This is an elegant approach where the label is incorporated directly into proteins as they are synthesized. Because stable isotope labeling facilitates multiplexing, quantitative differences attributed to run-to-run technical variation are eliminated

MS-ICT-100	ICAT™ experiment for 1 sample and 1 control
MS-ITRAQ-4	iTRAQ™ analysis 4 Plex
MS-ITRAQ-8	iTRAQ™ analysis 8 Plex
MS-MRM/DEV	Development for an MRM assay
MS-MRM/SRM	MRM/SRM sample analysis
MS-SILAC/DEV	Cell culture and Assay Development
MS-SILAC/MS	SILAC analysis 2-Plex

Post Translational Modification Analysis

Please enquire as there are numerous PTMs

Glycan Analysis



The addition of carbohydrate structures to recombinant glycoproteins is one of the most frequent protein modifications that is often essential for the protein to achieve full biological functionality and regulatory approval. APAF can now determine the N- and O-glycosylation of your recombinant protein and give information on the carbohydrate structural detail.

MS-GLY-100	Intact Mass including Glycans
MS-GLY-110	Intact mass after removal of N-Linked Glycans
MS-MLD-500	PMF ID glycoprotein
MS-OLIGO-100	Oligosaccharide analysis N-Linked
MS-OLIGO-150	Oligosaccharide analysis O-Linked
LC-DIONEX	Sugar/Carbohydrate analysis using Dionex LC

GC/MS for trace analysis

MS-GC-100*	GC/MS preparation and data acquisition
MS-GC-200	Database search/identification option

Software Platforms

APAF has a number of software platforms used in protein identification and 2D Gel Analysis. These include:

Progenesis PD Quest	Non Linear – gel imaging
Mascot	Bio-Rad – gel imaging
Protein Pilot	Matrix Science
Sequest	Applied Biosystems
X/Tandem	Thermo Finnigan
	Open Source

APAF is also a mirror site for the Swiss Institute of Bioinformatics

ExpASY Swiss Institute Bioinformatics

Bioinformatics

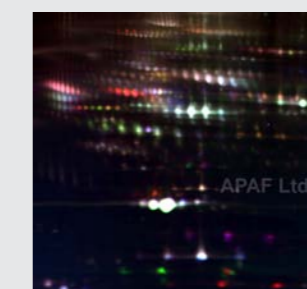
Having our own in-house scientists with proteomic, mathematic, and software development skills allows APAF to offer custom bioinformatic solutions. These services range from the simplistic such as the generation of custom reports and figures, to more complicated analysis such as the protein or peptide ratios that are statistically different from 1:1, and on the intensive end to the development of algorithms for the analysis of customer specific data. If you have a specific interest please do not hesitate to enquire.

Two Dimensional Gel Electrophoresis

High resolution Two-Dimensional Gel Electrophoresis (2DGE) is an integral tool in the study of Proteomics. Experiment design and sample preparation is done in close discussion with collaborators. Detailed image analysis of sample set expression profiles and automated recovery of proteins from gels followed by proteolytic digestion of proteins of interest for further analysis by mass spectrometry is also carried out.

APAF also offers Difference Gel Electrophoresis (DIGE) to our available proteomics services. In a DIGE system, proteins are pre-labelled with fluorescent CyDyes™ such as Cy2, (Internal Standard) Cy3, and Cy5 prior to electrophoretic separations. Labelled samples are then mixed before isoelectric focusing, and resolved on the same 2D gel thus removing gel-to-gel variation. Differentially expressed proteins are identified by an image analysis program.

Item No.	Description
2DG-M 11	2D Electrophoresis mini gel (11cm)
2DG-L 17	2D Electrophoresis large format gel (17cm)
2DG-L 24	2D Electrophoresis extra large format gel (24cm)
2DG-B 11	Blot from mini gel (Nitrocellulose or PVDF)
2DG-B 17	Blot from large format gel (Nitrocellulose or PVDF)
2DG-B 24	Blot from extra large format gel (Nitrocellulose or PVDF)
2DG-Q 50*	CyDye multiplexing for quantification of changes in Protein Levels between samples



DIGE gel after removal of high abundance plasma proteins

Peptide Immobilized pH gradient IEF

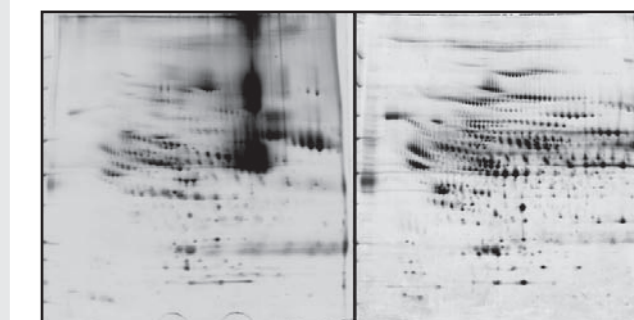
Peptide IPG-IEF separation can be a successful technique as the peptides are separated on IPG strips into discrete PI regions with high resolution. The peptides are then eluted from the strip and the resulting eluent assayed by either LC/MS/MS or LC/MALDI. For further information please enquire.

Sample Preparation

2DG-SP99	Sample preparation guidelines can be found at http://www.proteome.org.au and then follow to No. 1
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Removal abundant proteins

2DG-A 52	Analysis of low abundance proteins by 2DGE– requires removal of high abundance proteins.
APR-14AG	Removal of top 14 plasma protein human (Agilent)
APR-14BG	Removal of top 12 plasma protein human (Beckman)
APR-R7-300	Removal top 7 rodent plasma proteins



Gel comparison of with and without high abundance of protein removal.

Miscellaneous

FACH-24	Facility Hire Daily
FACH-72	Facility Hire Weekly
FACH-216	Facility Hire Monthly

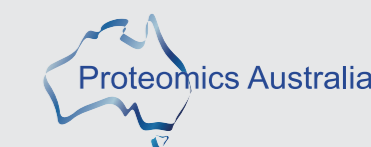
Courses

The courses will be available on a needs basis and run for three days. Courses are subject to change based on demand and availability

2DGEL	2D Gel Electrophoresis Course – Fees include all tuition, manual and materials
MS-000	Mass Spectrometer Introductory
MS-100	Mass Spectrometer Advanced

Structural Proteomics

PCD-60	CD Analysis
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High Throughput cell-based Screening

High Throughput cell-based Screening TGR BioSciences, a node of Proteomics Australia, offers a new luminescence based platform technology for measurement of cellular protein phosphorylation. Called SureFire®, the assays provide a quantitative measure of the degree of activation of key signalling pathways in cells. Since the assays are performed using living cells SureFire™ can be used to screen the activity of agents that work either on the outside of the cell or in the cytosol. SureFire® can be applied, for example, to the determination of receptor agonists and antagonists, and to assay the effect of small molecule inhibitors acting intracellularly. SureFire® is not restricted to cell type, and most mammalian cells can be used to examine the effects of test agents. These screening assays can be carried out on a fee-for-service basis at TGR or the kits can be purchased from TGR for use in-house by the client. The client will need access to an AlphaScreen® compatible plate reader. SureFire is a registered trademark of TGR Biosciences Pty Ltd. AlphaScreen is a registered trademark of PerkinElmer, Inc.

AlphaScreen® SureFire® Kinase Assays

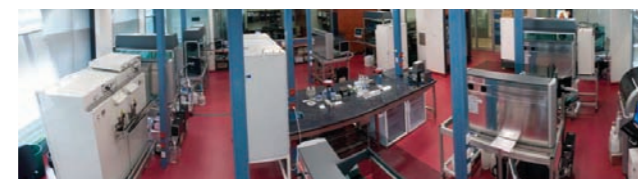
AlphaScreen® SureFire® p-Akt 1 (Thr308) 384 kit
 AlphaScreen® SureFire® p-Akt 1/2 (Ser473) 384 kit
 AlphaScreen® SureFire® p-BAD (Ser112) 384 kit
 AlphaScreen® SureFire® p-BAD (Ser136) 384 kit
 AlphaScreen® SureFire® p-Caspase9 (Ser196) 384 kit
 AlphaScreen® SureFire® p-ERK 384 kit
 AlphaScreen® SureFire® Total ERK 384 kit
 AlphaScreen® SureFire® p-GSK3α (Ser21) 384 kit
 AlphaScreen® SureFire® p-GSK3β (Ser9) 384 kit
 AlphaScreen® SureFire® p-IkKα (Ser32/Ser36) 384 kit
 AlphaScreen® SureFire® p-IkKβ (Ser177/Ser181) 384 kit
 AlphaScreen® SureFire® p-JNK 1/3 384 kit
 AlphaScreen® SureFire® p-MEK 1 384 kit
 AlphaScreen® SureFire® p-mTOR (Ser2481) 384 kit
 AlphaScreen® SureFire® p-NFκB p65 (Ser536) 384 kit
 AlphaScreen® SureFire® p-PDK-1 (Ser241) 384 kit
 AlphaScreen® SureFire® p-S6 RP (Ser235/236) 384 kit
 AlphaScreen® SureFire® p-S6 RP (Ser240/244) 384 kit
 AlphaScreen® SureFire® p-SMAD 2 (Ser465/467) 384 kit
 AlphaScreen® SureFire® p-p38 MAPK 384 kit
 AlphaScreen® SureFire® p-p70 S6 kinase (Thr229) 384 kit
 AlphaScreen® SureFire® p-p70 S6 kinase (Thr389) 384 kit
 AlphaScreen® SureFire® p-p70 S6 kinase (Thr421/Ser424) 384 kit
 AlphaScreen® SureFire® p-Stat-3 384 kit
 AlphaScreen® SureFire® p-Stat-5A/B (Tyr694/Tyr699) 384 kit
 AlphaScreen® SureFire® p-4E-BP1 (Thr37/Thr46) 384 kit
 AlphaScreen® SureFire® p-4E-BP1 (Thr70) 384 kit

Further assays are under development please inquire.

Monash Antibody Technologies Facility

The MATF is one of the only high throughput laboratories in the world offering custom-made, high-affinity antibodies. The combination of full robotic cell culture, innovative antigen treatment and novel, proprietary screening allows the facility to generate thousands of new monoclonal antibodies per year. The MATF removes the obstacles researchers in academia and industry face when trying to source quality antibodies. MATF is an open-access facility providing international scientists with superb quality anti-protein and anti-peptide monoclonal antibodies and related services. We welcome challenging projects, PTM-specific targets and comprehensive projects involving families of antibodies.

MATF-100	Generation of monoclonal antibodies
MATF-200	Anti-recombinant protein antibodies
MATF-300	Anti-synthetic peptide antibodies
MATF-320	Peptide Design
MATF-350	Peptide coupling to carrier
MATF-400	Antibody purification

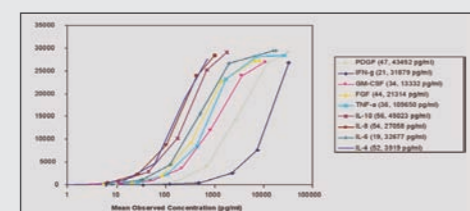


Multiplexing Technologies

Bead based multiplexing assays are available from a number of sources covering many analytes. APAF on the Bio-Plex instrument and robotic liquid handling systems, is able to run your assays at a fixed cost per plate plus kit and consumable costs.

The system enables you to multiplex (simultaneously measures) up to 100 analytes (peptides, proteins, DNA etc) in a single well, using very small sample volumes, allowing quick and cost effective bioassays, while ensuring optimum accuracy.

xMAP- Kit	kit and consumables
xMAP- Assay	Assay of 96 well plate
xMAP-EpRo	Robot consumables



Standard curve assay for a 9 – Plex cytokine assay

Note: Legend - Analyte (bead region, concentration of standard 1).
 • Subsequent standard vials were diluted four times.

Bioplatforms Australia

Bioplatforms Australia was established to manage an Australian Government investment through the National Collaborative Research Infrastructure Strategy (NCRIS) with support from State Governments, Academic and Research Institutes and commercial entities.

The Bioplatforms Australia investment is aimed at supporting infrastructure and expertise across the fields of:

Proteomics	-	Proteomics Australia
Genomics	-	Genomics Australia
Metabolomics	-	Metabolomics Australia
Bioinformatics	-	Australian Bioinformatics Facility

Proteomics Australia underpins Australian life science research and contributes to national research priorities through provision of world

class proteomics infrastructure, expertise and advanced protein discovery services across the life sciences, agri-food and human health sectors. Included within this group are the following nodes:

- Australian Proteomics Analysis Facility (APAF)
- The Bio-analytical Mass Spectrometry Facility (BMSF)
- TGR BioSciences
- Monash Biomedical Proteomics Facility
- Monash Antibody Technology Facility
- Queensland Institute of Medical Research

Proteomics Australia is a newly formed consortium of peak proteomics service providers from around Australia with synergistic expertise. All infrastructure and expertise will be made openly accessible to Australian and international researchers from academia, publicly funded research institutes and the commercial sector alike.

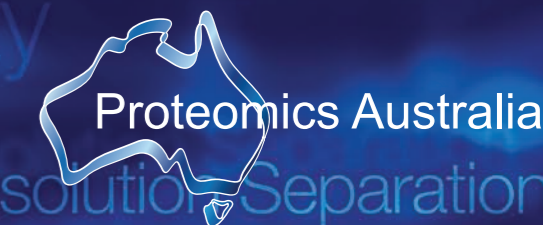


Mass Spectrometry
 Protein Profiling



LC-MALDI

BioActivity



High Resolution Separation

Bioinformatics

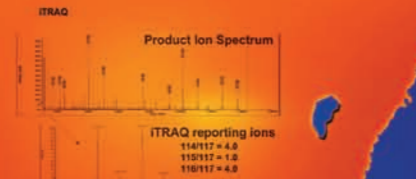
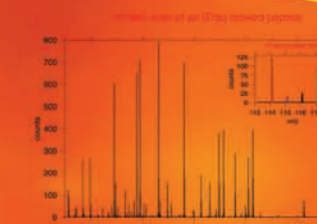
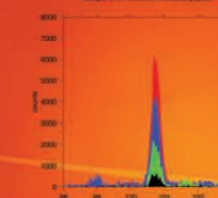
The Australian Proteome Analysis Facility

Identification

A Service and Discovery Facility

MRMS

SILAC



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ESI-MS-MS
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