



Proteomics Services

Accredited Protein Analytical Services

Biosimilars / Biologics, proteins and peptides

We work with **Proteomics International** for the delivery of contract research and lead molecule discovery services in proteins, peptides and biopharmaceuticals. **Proteomics International** was established in 2001 and is ISO 17025 accredited for proteomics testing.

Our suite of Proteomics Services includes high quality protein identification, analytical and characterisation services utilizing the most advanced high throughput mass spectrometry instrumentation (MALDI TOFTOF, LC/MS/MS and ion trap).

Proteins can be fully characterised to meet your research or development needs.

A Complete Range of Services

Mass Spectrometry Services

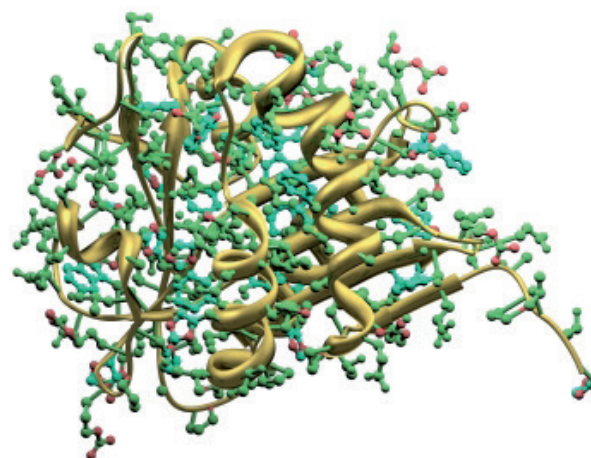
- Protein Identification
- Protein Sequencing by Mass Spectrometry (MALDI TOF, LC/MS)
- *De novo* peptide sequencing
- Peptide mass fingerprinting
- Mass determination of intact proteins and peptides

Proteome Mapping

- MudPit Analysis
- Differential Expression (using iTRAQ)

Other Services

- Amino Acid Analysis
- Quantitation Analysis (by MRM)
- Phosphorylation Detection
- N-terminal sequencing
- Biosimilars / Biologics Drug Characterisation (please enquire)
- Biomarker Discovery (please enquire)
- Pharmacokinetic Testing (please enquire)



Protein Identification Analysis

For protein sequencing mass spectrometry, specifically tandem MS (MALDI-TOF/TOF or electrospray MS/MS), is used.

Service Features:

- Routinely protein identification analysis is offered from any source - gels, liquids, and dry samples.
- Analysis by MALDI-TOF/TOF (PMF+MS/MS) is optimal for Coomassie stained gels and 2D gel spots.
- Analysis by Electrospray (LC/MS/MS) mass spectrometry is optimal for gel bands containing two or more proteins or lower abundant samples, e.g. 1D gel band containing multiple proteins or 2D gel spots with silver staining. Sample pooling is required for silver or fluorescent stained spots.
- Protein identification is achieved by matching the peptide fragmentation pattern to theoretical sequences in the public databases. This is ideal for human, mouse, rice, arabidopsis, fruit fly, etc. If the samples are from organisms highly similar to known genomes then good results may be achieved.

Deliverables:

The end result is positive verification of protein identity.
Time: 2 weeks upon arrival of order at analysis laboratory

Limitations:

- For samples which cannot be identified by automatic database analysis, *de novo* peptide sequencing analysis will be performed.

| Product No. | Name of Service | Service Includes | Suited for |
|--|---|--|--|
| Protein Identification Services | | | |
| SS001-1 | MALDI-TOF/TOF mass spectrometry (Sequencing by MS/MS) | Trypsin digestion of protein, running of samples with automatic database analysis. | Optimal for pure protein from 2D gel spots. |
| SS001-2 | Electrospray (LC/MS/MS) Mass Spectrometry | Trypsin digestion of protein, running of samples with automatic database analysis. | Optimal for a single gel band containing 2 or more proteins, or low abundant samples, or proteins <20kDa |

De novo Peptide Sequencing Analysis

De novo sequencing is only performed when the protein sample is not available in public databases. This can occur when the genome of the organism is not complete. If the protein is available in public databases then Service 001 is performed with Mascot database searching.

Service Features:

- Sample for *de novo* sequencing is prepared as per Service 001, only the data interpretation is different.
- Tandem mass spectrometry (MALDI-TOF/TOF or electrospray MS/MS) is used to determine the amino acid sequence of proteins and peptides that are not present in currently available databases.
- The sample spectra are interpreted to obtain *de novo* protein sequence for each peptide ion. This is the equivalent of N-terminal Edman sequencing of internal peptides and, if successful gives the highest quality data. The sequences can range in length from 6-20+ amino acids and these can be used to design an oligo probe.
- Gels and lyophilised samples can be used for *de novo* sequencing analysis.

Deliverables:

The results provided are a short peptide sequence, normally 8-15 amino acids. The Client must then BLAST search this sequence against public databases to find **homologous** protein matches. Multiple peptides may be *de novo* sequenced if required. Normally 3-5 peptides are sufficient to determine functional homology, or to design an oligo probe.

Time: 2 - 3 weeks upon arrival of order at analysis laboratory

(This is based on the standard ProID service 001, PLUS additional data analysis using *de novo* sequencing tools.)

Limitations:

The Client must BLAST search the sequence against public databases to find homologous protein matches.

| Product No. | Name of Service | Service Includes | Suited for |
|--|--|--|--|
| Protein Identification Services | | | |
| SS002-1 | <i>De novo</i> Peptide Sequencing by Mass Spectrometry. Only available for a single pure protein or protein gel band stained with Coomassie blue, that does not have the related genome from database. Price per sample with 1 peptide sequence for <i>de novo</i> search. | MALDI-TOF/TOF or LC/MS/MS is used to determine the primary sequence structure of proteins. Manual interpretation of the MS/MS spectra and derive of internal sequence will be done by the expert operator using the latest software. | Only available for a single pure protein or protein gel band stained with Coomassie blue, that does not have the related genome from database. |
| SS002-2 | <i>De novo</i> Peptide Sequencing by Mass Spectrometry. Only available for pure protein stained with Silver or other MS compatible stains, that does not have the related genome from database. Price per sample with 1 peptide sequence for <i>de novo</i> search. | | Gels with Silver stain or other MS compatible stain e.g. Ruby or Cy dyes, that does not have the related genome from database. |
| SS002-3 | <i>De novo</i> Peptide Sequencing, each additional peptide sequence for <i>de novo</i> search. | For <i>de novo</i> samples that need to look for probe/primer design, minimum 3 peptide sequences for <i>de novo</i> search are recommended. | Optimal for pure protein from 2D gel spots. |

Note: While sample's workflow for *de novo* sequencing is the same as for Service 001 Protein Identification by MALDI ToF/ToF or Electrospray (LC/MS/MS), it differs in the data interpretation approach.

Proteome Mapping or MudPit (Multi-Dimensional Protein Identification Technology) Analysis

The proteome is analysed by LC-MS: a combination of chromatography separation and mass spectrometry.

Service Features:

- The identification of large numbers of proteins through proteome mapping experiments is possible with the use of two dimensional chromatographic separations and the sensitivity of modern mass spectrometers.
- In comparison to the 10s to 100s of protein identifications possible from 2D gel electrophoresis, the use of 2D LC-ESI based systems provides 100s to 1000s of protein hits in a timely and cost effective manner.
- From 10 µg of protein (proteome) a simple 1D LCMS experiment can identify 10s to 100s of proteins. With 50-200 µg of protein (proteome) a full 2D LCMS mapping experiment can be undertaken to identify 100s to 1000s of proteins.
- Essentially any protein sample such as cell lysate, whole tissue and secreted proteins may be analysed.

Deliverables:

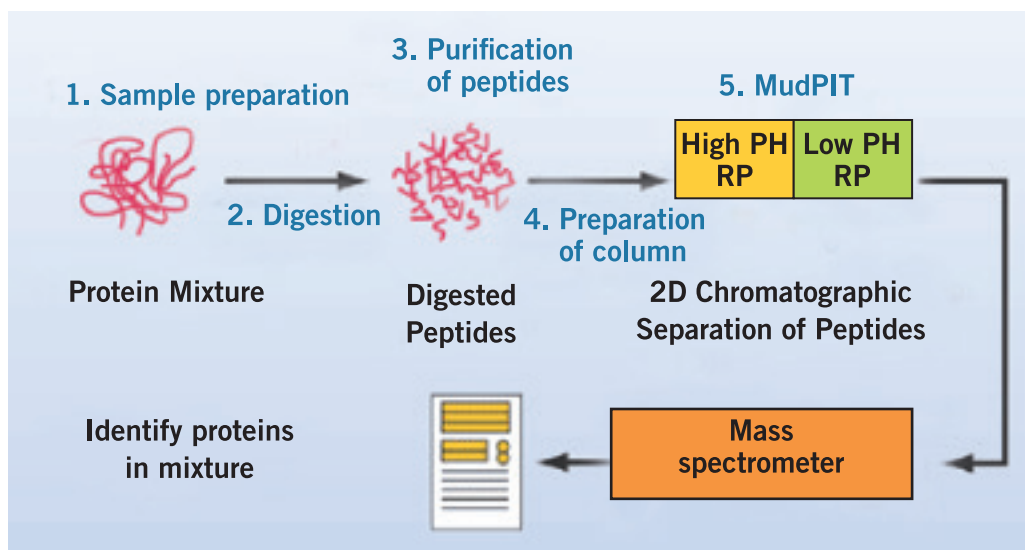
The number of all proteins identified.
Time: 2-3 weeks upon arrival of order at analysis laboratory

Limitations:

- Proteome mapping analysis works best on samples from a known genome.
- Where genomes are partially known the normal mapping approach will give limited results. However, Proteomics International's advanced *de novo* sequencing techniques may be applied.

Workflow

MUDPIT (Multidimensional Protein Identification Technology)



| Product No. | Name of Service | Service Includes | Suited for |
|---|---|--|--|
| Proteome Mapping – MuDPIT Analysis | | | |
| SS003-1 | Proteome Mapping – MuDPIT analysis: 1D LC-MS. Price per Sample | Simple protein sample is run through an extended LC gradient and eluted into the LC-ESI. | Optimal for simple protein mixtures, allows identification up to 100 protein per experiment. |
| SS003-2 | Proteome Mapping – MuDPIT analysis: 2D LC-MS. Price per Sample. | Complex protein sample is run through a sophisticated 2D LC gradient and eluted into the LC-ESI. | Optimal for complex protein mixtures, allows identification up to 1000 protein per experiment. |

Differential Expression Analysis by iTRAQ

This service gives data on the relative and absolute quantitation of proteins and peptides.

Service Features:

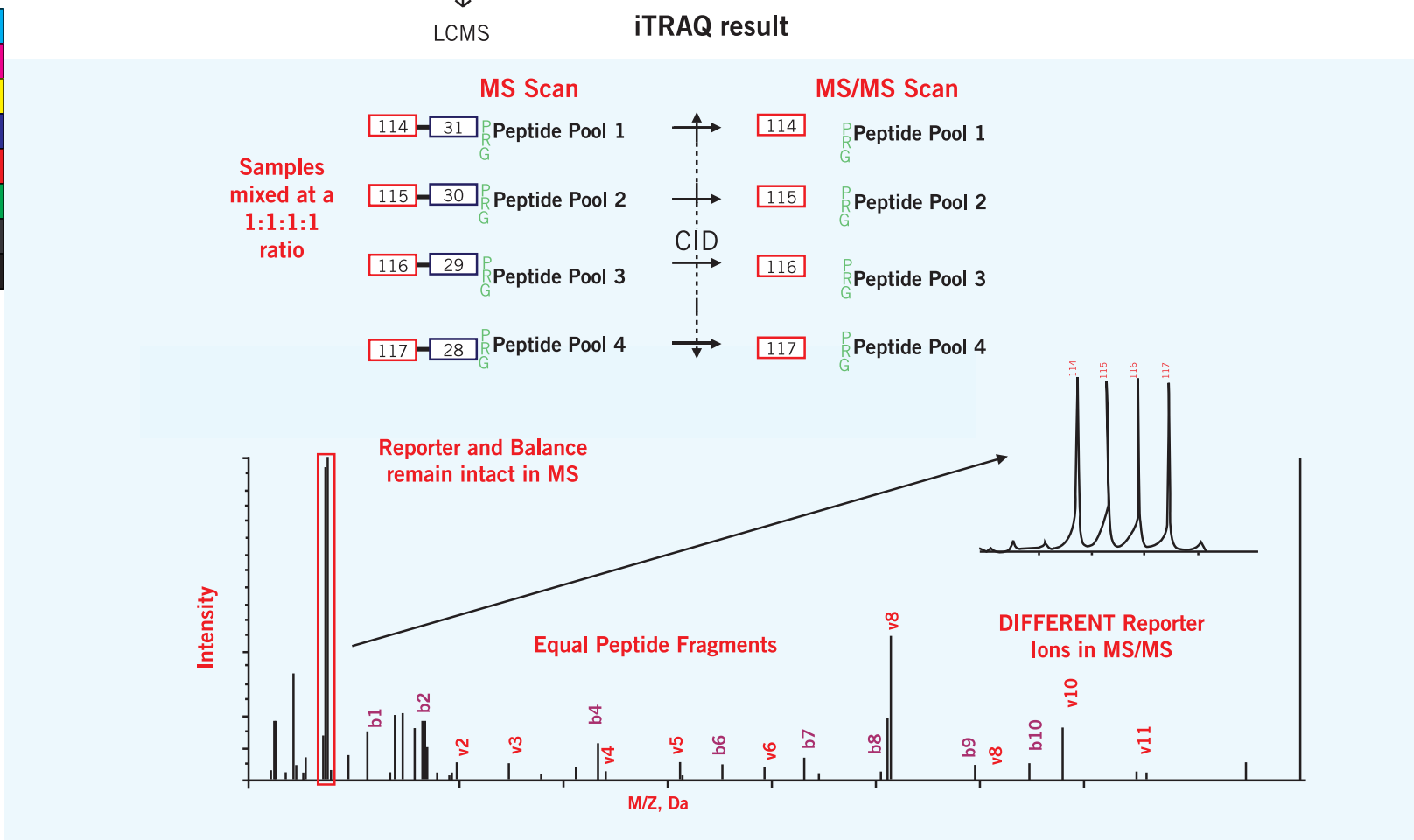
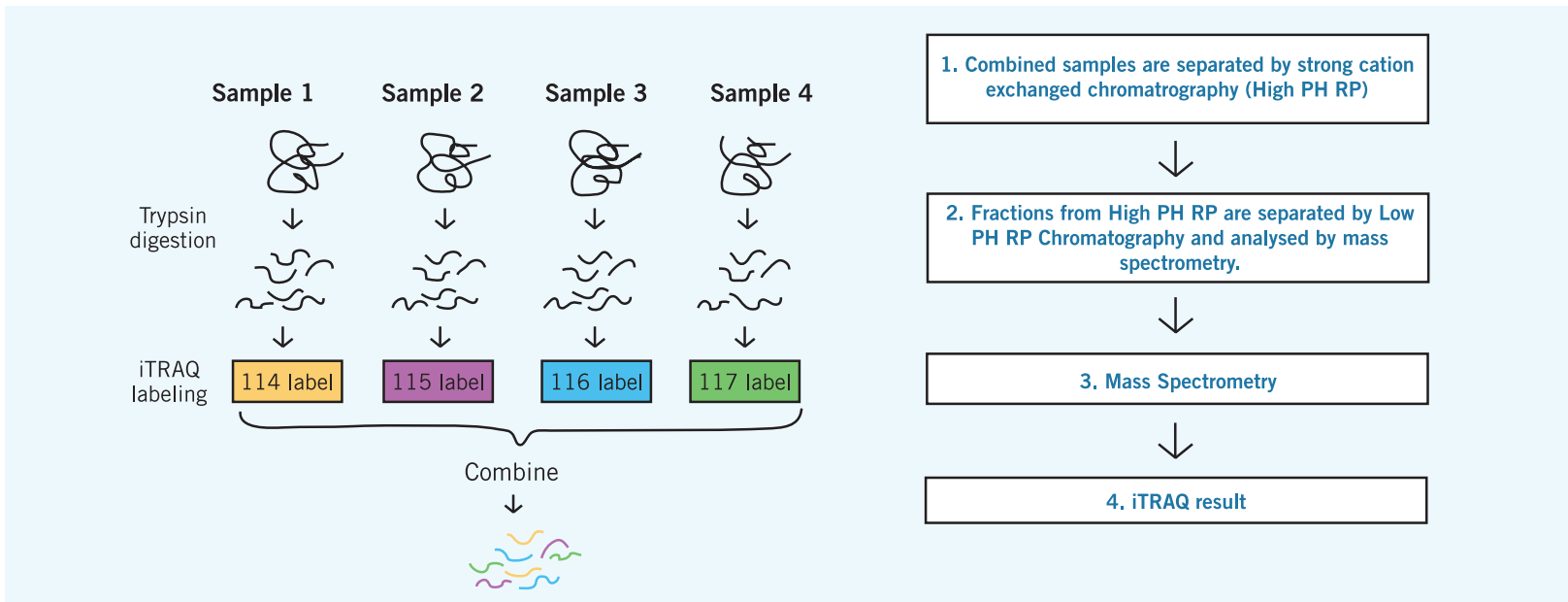
- *iTRAQ enables relative and absolute quantitation of protein and peptides by labelling samples with isotope encoded reporter ions.*
- *Up to 4 or 8 different proteome samples may be labeled with iTRAQ reagents and compared simultaneously.*
- *A Quantitative Proteome Mapping experiment is the best way to provide differential protein expression information within a wide range of biological cell types and systems.*
- *The optimal amount for an iTRAQ experiment is 50 µg tagged protein mixture. To ensure sufficient protein is available after clean-up ~250 µg protein per sample is required for labeling for 2 tag, ~150 µg per sample is adequate for 4 tag. The minimum specifications are stipulated because up to 80% of sample may be lost during sample cleanup. Greater amounts of total protein will improve sensitivity towards low abundance proteins; lower quantities may provide sub-optimal results*
- *Essentially any protein sample such as cell lysate, whole tissue, and secreted proteins may be analysed.*
- *The 4-plex iTRAQ kit is used because of the high quality of data obtained, rather than the 8-plex iTRAQ kit because of emerging evidence that the latter produces comparatively poor proteome coverage*

Deliverables: Data will provide the number of all proteins identified and their relative concentrations.
Time: 3-4 weeks upon arrival of order at analysis laboratory

Limitations: iTRAQ analysis will not work on samples from an unknown genome.

Workflow

iTRAQ 4-plex experiment



| Product No. | Name of Service | Service Includes | Suited for |
|---|--|---|------------|
| Protein Differential Expression – iTRAQ Analysis | | | |
| SS005-1 | Differential expression – iTRAQ analysis service. Price for 1st experiment (up to 4 samples) | Sample labeling and analysis by 2D LC-MS mass spectrometry, with automatic database analysis. | |

| Product No. | Name of Service | Service Includes | Suited for |
|---|--|--|---|
| Quantitation Analysis | | | |
| SS006-1 | Multiple Reaction Monitoring (MRM) analysis: Method development. Price per day. | Estimated 2 to 5 days are required for each method. | Biomarker analysis and reaction monitoring in pharmacokinetic studies for toxic metabolites. |
| SS006-2 | Multiple Reaction Monitoring (MRM) analysis: Data collection and analysis. Price per sample. | Data collection and analysis performed as agreed in the project/ method from SS006-1. | Recommended for quantification of proteins, peptides, metabolites and lipids from plasma, serum and other biological samples. |
| Phosphorylation Detection Services | | | |
| SS007 | Phosphorylation Detection. Price per sample. | Precursor ion and neutral loss scans that allow detection of the loss of the phosphate group. | Identification of phosphorylation sites may be mapped to detect the important post-translational modification of proteins. |
| Protein Mass Analysis | | | |
| SS008-1 | Protein Mass analysis: peptide (<6 kDalton). Intact mass by MALDI-TOF mass spectrometry. Price per peptide. | Samples are processed intact (no enzyme digestion). Intact mass by MALDI-TOF mass spectrometry. | Pure peptide/protein (<6 kDalton). |
| SS008-2 | Protein Mass analysis: protein (<6 kDalton). Intact mass by MALDI-TOF mass spectrometry. Price per protein. | | |
| SS008-3 | Protein Mass analysis: peptide (>6 kDalton). Intact mass by electrospray mass spectrometry. Price per peptide. | Samples are processed intact (no enzyme digestion). Intact mass by electrospray mass spectrometry. | Pure peptide/protein (>6 kDalton). |
| SS008-4 | Protein Mass analysis: protein (>6 kDalton). Intact mass by electrospray mass spectrometry. Price per protein. | | |
| Component Analysis | | | |
| SS009-1 | Amino Acid Analysis: Peptide. Price per sample. | Chemical hydrolysis up to 18 amino acids per analysis. Excludes Cystein and Tryptophan detection. | Ideal for quality control and the amino acid composition of peptides. |
| SS009-2 | Amino Acid Analysis: Protein. Price per sample. | | For protein and peptide samples (include recombinant proteins from cell culture and fermentation material, nutraceuticals and plant materials). |
| SS009-3 | Amino Acid Analysis: Determination of Cystein per analysis. Price per sample. | Special hydrolysis method to detect only Cystein. | |
| SS009-4 | Amino Acid Analysis: Determination of Tryptophan per analysis. Price per sample. | Special hydrolysis method to detect only Tryptophan. | |

Proteomics International's High Level of Expertise

Contract research services are routinely provided by **Proteomics International** to **identify molecules, investigate differential expression and to fully characterise proteins, peptides and biosimilar drugs**. All work is performed to a high standard from scientists' expert in their field using ISO 17025 accredited procedures and is treated as confidential.

Consultative approach - Full project consultation is provided including multiple services in one package covering all your requirements within the one CRO. Our scientists work with you so that you achieve the **best outcomes** from your discovery or development projects.

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<http://www.proteomics.com.au/>

For more information on how to submit your samples for analysis, please visit:

<http://base-asia.com/proteomics-services/sample-preparation>

or

<http://www.base-asia.com/proteomics/how to order>



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