

SERVICES DESCRIPTION

1. Protein quantitation & 1D-SDS-gel electrophoresis of suitable liquid sample

- Protein quantitation by BCA protein assay and single mini-gel 1D PAGE

2. Mass spectrometry based protein identification of individual coomassie-blue stained band(s) from 1D or 2D-SDS-Gels

- Cut out bands and document analysis
- Tryptic digestion
- Mass spectrometry based protein identification and analysis (option 1-5)

Options:

- 1). High-throughput single algorithm search (Maldi QStar peptide MS/MS analysis with automated single database search and expert data report supplied)
- 2). High-throughput single algorithm search (Maldi QStar peptide MS/MS analysis with automated single database search for user identification)
- 3). Extended analysis single algorithm search (peptide separation (μ HPLC) ESI peptide MS/MS analysis with automated single database search and expert data report supplied)
- 4). Extended analysis single algorithm search (peptide separation (μ HPLC) ESI peptide MS/MS analysis with automated single database search for user identification)
- 5). Custom analysis extensive peptide survey (peptide separation (μ HPLC) ESI peptide MS/MS analysis with automated database search(s) and extensive manual data-mining (*De-novo* analysis etc.) and expert data report supplied.

For all communications, email proteomics@ludwig.edu.au

3. Mass spectrometry based identification of liquid protein mixtures

For whole protein MW analysis

- Sample concentration and buffer exchange
- Mass spectrometry analysis by ESI-MS analysis (protein separation by μ HPLC).

For peptide analysis from whole protein

- Sample concentration, buffer exchange, reduction and alkylation and tryptic digestion
- Mass spectrometry analysis (options 1-5)

Options:

- 1). High-throughput single algorithm search (Maldi QStar peptide MS/MS analysis with automated single database search and expert data report supplied
- 2). High-throughput single algorithm search (Maldi QStar peptide MS/MS analysis with automated single database search for user identification
- 3). Extended analysis single algorithm search (peptide separation (μ HPLC) ESI peptide MS/MS analysis with automated single database search and expert data report supplied
- 4). Extended analysis single algorithm search (peptide separation (μ HPLC) ESI peptide MS/MS analysis with automated single database search for user identification
- 5). Custom analysis extensive peptide survey (peptide separation (μ HPLC) ESI peptide MS/MS analysis with automated database search(s) and extensive manual data-mining (*De-novo* analysis etc.) and expert data report supplied.

4. 2D-Size-Exclusion chromatography and 1D-Gel (mini SDS-PAGE) separation of protein mixtures

- Sample concentration (if required)
- SEC separation in preparation for 2nd dimension
- 1D-SDS-Gel Analysis (coomassie-blue stained and imaged)
- For identification of coomassie-blue stained proteins, choose option from Item #2

5. 2D-Gel (mini IEF:SDS-PAGE) separation of protein mixtures

- Sample concentration (if required)
- IEF gel separation in preparation for 2nd dimension
- 2D-SDS-Gel Analysis (coomassie-blue stained and imaged)
- For identification of coomassie-blue stained proteins, choose option from Item #2

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6. N-Terminal Sequencing (external service)

Sample Preparation

- Samples should contain a minimum of 2 picomoles of the protein/peptide to be sequenced.
- Protein can be submitted dry, in solution, or blotted to PVDF membrane.
- Nitrocellulose is not compatible with the Edman chemistry.

Dry Sample

The sample will be reconstituted in 0.1% TFA/20% acetonitrile so it should be soluble in this buffer. If you think there will be a solubility problem please contact us.

In Solution

Sample should be free from interfering buffers and salts. Buffers containing primary amines, such as TRIS and HEPES, should in particular be avoided, although low concentrations of SDS (up to 0.3%) and PBS can be tolerated. If interfering buffers/salts are unavoidable it should still be possible to sequence the material after clean up. We ask that a minimum of 10 picomole be supplied if clean-up is required.

On PVDF Membrane

In general, best results are obtained using 2D gels blotted to PVDF. The protein can be stained with Coomassie Blue R-250, Amido black, Ponceau S or Sypro Ruby. Do not use silver stain. Other stains may be compatible but please contact us prior to use.

7. Amino acid analysis (external service)

Amino Acid Analysis

Sample Preparation

- The sample should contain 5 micrograms or more of the protein to be analysed.
- The sample can be submitted dry, in solution or blotted onto PVDF membrane.
- Purity of the sample is critical. The following types of contamination will interfere with the analysis: proteins, amino acids such as glycine and arginine, buffers and salts. A 10% protein contamination can make your results meaningless. Tris, HEPES, glycerol and other primary and secondary amines must be avoided completely.
- Low molecular weight solutes can be removed by dialysis (if you have sufficient protein), by reversed phase HPLC or by loading onto a ProSorb filter (PE Biosystems) and washing the PVDF membrane well with 0.1% TFA.
- For best results samples should be supplied in Milli-Q water or dried. If your protein is not readily soluble, please inform us as this may greatly influence quantitation.

NOTE:

- Acid hydrolysis converts asparagine and glutamine to aspartic and glutamic acid respectively. That is, the amino acid analysis result for Asp is a total of Asp + Asn and the result for Glu is Glu + Gln.
- Tryptophan is destroyed by acid hydrolysis and requires a separate analysis using base hydrolysis.
- Cys determination also required a separate analysis. It is analysed as cysteic acid after oxidation with performic acid.
- For unusual amino acids, such as hydroxyproline and taurine please contact us.

8. Consultation on proteomics

- The facility is able to train personnel in proteomics related techniques to enhance the prospective research group for generation of samples for analysis by the facility. Please contact us to tailor your specific requirements.

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EXPECTED PROCESSING TIME (DAYS)*

1. Protein quantitation & 1D-SDS-gel electrophoresis of suitable liquid sample

2 days

2. Mass spectrometry protein identification of individual coomassie-blue stained band(s) from 1D or 2D-SDS-Gels

Options

- | | |
|---|---------------------------|
| 1 & 2). High-throughput single algorithm search | 3 days (≤ per 20 samples) |
| 3 & 4). Extended analysis single algorithm search | 5 days (≤ per 10 samples) |
| 5). Custom analysis extensive peptide survey | (by consultation) |

3. Mass spectrometry protein identification of liquid protein mixtures

For whole protein MW analysis

ESI or Maldi-Tof-MS analysis

2 days (≤ per 10 samples)

For peptide analysis from whole protein

Options

- | | |
|---|---------------------------|
| 1 & 2). High-throughput single algorithm search | 3 days (≤ per 20 samples) |
| 3 & 4). Extended analysis single algorithm search | 5 days (≤ per 10 samples) |
| 5). Custom analysis extensive peptide survey | (by consultation) |

4. 2D-SEC and 1D-Gel (mini SDS-PAGE) separation of protein mixtures

7 days

For identification of coomassie blue stained proteins see Item #2.

5. 2D-Gel (mini IEF:SDS-PAGE) separation of protein mixtures

7 days

For identification of coomassie blue stained proteins see Item #2.

6. Amino acid analysis (external service)

7-14 days

7. N-Terminal Sequencing (external service)

7-14 days

8. Consultation on proteomics

by appointment

NB* Sample turnaround may be affected by our workload, please check when submitting samples