

Faculté de biologie et de médecine

Protein Analysis Facility

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Services and Fees

IMPORTANT :

- * "Lausanne academic" includes UNIL, CHUV, EPFL, Ludwig institute
- * Mass spec/protein ID : all prices are intended per sample submitted, in swiss francs (CHF)
- * Gel separation : all prices are intended per gel in swiss francs (CHF)
- * For simultaneous submission of 10 or more samples a 10% discount on total cost is applied
- * All prices can be subject to change without prior notice

[Click on the description for more details on every service workflow offered](#)

MASS SPECTROMETRY and PROTEIN IDENTIFICATION

No.	Description	Applicability	Lausanne academic	External academic	Private (commercial)
1	Protein identification by MALDI (MS and MS/MS)	only spots from 2D gels	40	90	150
2	Protein identification by short LC-MS run	simple mixtures, samples from 1D gel bands or 2D gel spots	80	150	220
3	Protein identification by nanoLC-MS/MS : long LC-MS run	1D gel bands for in-depth analysis (PTMs); medium complexity mixtures; max sensitivity	160	320	500
4	Direct protein identification by nanoLC-MS/MS	medium complexity mixtures; only for samples ready to inject (requires setup tests)	100	not done	not done
5	Special data analysis (per project *day)	customized database setup, post-translational modifications, validation, etc	200	250	500
6	Direct MALDI analysis	protein/peptide intact mass	60	150	ask us
7	Direct electrospray analysis	protein/peptide intact mass	60	150	220
8	Sample preparation (desalting...)	clean up before analysis (often necessary for 6,7)	60	150	220
9	Phosphorylation site analysis	determine site of protein modification	700	1200	2000
10	Shotgun analysis of complex samples	protein complexes or subcellular fractions	700	1200	2000
11	SILAC workflow (12 fractions)	quantitative proteomics of complex samples from mammalian cell cultures	1800	3000	ask us
12	Multiplex quantitation (iTRAQ)(12 fractions)	quantitative proteomics of any type of complex samples ; up to 8 samples	2300	ask us	ask us
13	SILAC culture media	RPMI or DMEM for SILAC (incl. labelled amino acids)	ask us	ask us	not done

14	<u>Supplement for high resolution fractionation (24 fractions)</u>	very complex mixtures - get the maximum data	900	1500	not done
GEL-BASED PROTEIN SEPARATIONS AND PROFILING					
15	<u>1D gels + Coomassie or Silver staining (minigels)</u>	simple protein mixtures, sample preparation for MS	60	100	180
16	<u>2D gels + Coomassie staining (minigels)</u>	simple protein mixtures	80	150	250
17	<u>2D gels + Silver staining (minigels)</u>	simple protein mixtures in low concentration	80	180	300
18	<u>2D gels + Western transfer (minigels)</u>	specific detection	100	200	400
19	<u>2D gels + Coomassie staining (midigels 13x9 cm)</u>	convenient for general screening purposes	90	180	300
20	<u>2D gels + Coomassie staining (maxigels 18x20cm)</u>	detailed analysis	200	400	ask us
21	<u>2D gels + DIGE* (all sizes)</u>	accurate quantitation and differential analysis	600	1200	ask us

* - Differential In Gel Electrophoresis

Protein identification by MALDI (MS and MS/MS) : starting from 2D gel spots, proteins are digested and peptides extracted. The unfractionated mixture is analysed on a 4700 MALDI-TOF-TOF and includes peptide mass measurement and MS/MS fragmentation on the ten most intense peptide peaks. Note : silver stained gel slices are accepted only if an MS-compatible staining protocol has been used.

Protein identification by short LC-MS run: samples, spots from 2D gels or bands from 1D gels of low complexity mixtures are digested and peptides are analysed by LC-MS/MS on a fast gradient.

Protein identification by nanoLC-MS/MS (long run) : starting from gel bands or LC fractions, proteins are digested and peptides extracted. The mixture is then separated on a nano-HPLC system on-line to an electrospray mass spectrometer, which isolates and fragments as many peptides as possible during a 30- to 90-min gradient. Collections of MS/MS spectra are used for database search for protein identification. This workflow is used for analyzing simple to medium complexity mixtures (typically 50-100 proteins) as well as for searching for modified peptides (PTMs). It is also recommended for samples from species with incomplete genome sequence information available.

Direct protein identification by nanoLC-MS/MS (samples ready to inject) : same as above but for large projects in which the customer performs sample preparation so that samples are ready to inject. Workflow and final sample quality are subject to test before acceptance

Special data analysis (per project) : includes all project specific data analysis procedures, such as setup and formatting of a special sequence database (for example organism-specific) or special database searches with one or more post-translational modification and validation of the obtained hits. Does not include peptide and/or protein quantification.

Direct MALDI analysis : a peptide or small protein is analysed directly by MALDI to obtain its accurate molecular weight. Usually works well only for masses up to 30'000 Da. Requires concentrated samples.

Direct electrospray analysis : a peptide or small protein is analysed directly by electrospray-MS to obtain its accurate molecular weight. Usually works well only for masses up to 30'000 Da. Requires concentrated and

detergent-free samples.

Sample preparation (desalting or other) : whenever necessary, a sample preparation step before MS can be performed by off-line liquid chromatography (reversed-phase or cation exchange) to clean up the sample. Often required for Direct MALDI/Direct Electrospray analysis.

Phosphorylation site analysis : gel-separated or liquid phase samples are digested and phosphopeptides are enriched by an affinity step on a titanium dioxide microcolumn. The eluate from the TiO₂ column is analysed by nano-LC-MS/MS to identify a maximum of phosphorylated peptides. This is a comprehensive package including one repetition of experiments for confirmation as well as, if needed, digestion with two different enzymes (for example trypsin and chymotrypsin) for better sequence coverage. Notes : an appropriate negative control (non phosphorylated) should be supplied whenever possible. The price is per sample. If needed, additional gel separations are billed.

Shotgun analysis of complex samples : protein mixtures are separated by limited electrophoresis after which 3-5 molecular weight regions are cut and digested. Analysis is performed by LC-MS/MS on every fraction. The resulting collections of spectra are pooled for every sample before database search. Lists of identified proteins for each sample with their scores are subjected to statistical validation and aligned for comparison. Note : for the analysis of protein complexes, a negative control is essential for background subtraction.

SILAC workflow : SILAC is an accurate quantitative proteomics technique based on stable isotope labelling with amino acids in cell culture. It is mostly only applicable to mammalian cultured cells but offers great advantages for experiments in which complex purification steps are required to isolate a fraction of interest, as well as for general quantitative profiling. The PAF offers support during the setup of SILAC labelling experiments as well as the full analytical pipeline (including data analysis). However, carrying out cell culture and the first steps in sample preparation remain responsibility of the customers. Preliminary in-depth project discussion is mandatory. Contact us for more information.

Multiplex quantitation (iTRAQ)(12 fractions): iTRAQ labelling is used for multiplex relative quantitation of up to 8 samples in the same experiment. Labelling is carried out at the peptide level after protein digestion and any type of proteomics sample (cell culture, tissue, etc.) can be quantitated by this technique. The workflow includes data analysis. Preliminary project discussion is mandatory.

SILAC culture media: the PAF can provide users with all reagents necessary to SILAC cell culture : special media (DMEM or RPMI), dialysed serum, isotope labelled amino acids. Exact prices are based on amount necessary and isotope labels chosen. Details have to be discussed, please contact us.

Supplement for high resolution fractionation (24 fractions): for very complex samples in SILAC or iTRAQ experiments, additional fractionation is recommended for higher proteome coverage.

1D gels + Coomassie or Silver staining (minigels) : simple separation on standard minigels

2D gels + Coomassie staining (minigels) : mostly used for preliminary tests to optimize sample preparation and migration conditions

2D gels + Silver staining (minigels) : mostly used for preliminary tests to optimize sample preparation and migration conditions. For maximum sensitivity silver staining is used. Not recommended for subsequent MS analysis

2D gels + Western transfer (minigels): for western blotting applications, 2D separation on a minigel is often sufficient. The service we provide includes transfer onto nitrocellulose, after which the membrane is returned to the user for detection with antibodies.

2D gels + Coomassie staining (midigels 13x9 cm) : used for preliminary tests of migration. Can also be used to perform preliminary DIGE experiments. We use precast gradient gels in the second dimension, which provide

very good separations despite their relatively small size.

2D gels + Coomassie staining (maxigels 18x20cm) : general purpose gels for separation of complex mixtures. Migration of samples on different gels limits reproducibility especially for weak spots. Basic image comparison is included.

2D gels + DIGE* (midi- and maxigels) : to overcome problems due to the limited reproducibility of 2D-electrophoresis, two samples A and B to be compared are labeled with different fluorophores (Cy3 and Cy5), then mixed and separated on the same gel. A third sample prepared by mixing 1:1 samples A and B is labeled with Cy2 and co-migrated as well as an internal standard. The gel is then scanned at three fluorophore-specific wavelengths to image all the samples. No image warping and matching is required since migration is in principle identical. Quantification is greatly facilitated by the use of the internal standard. We recommend this workflow for all demanding applications where accurate quantification and sensitivity is required. Unfortunately the Cy dyes are very expensive, which makes this type of analysis quite costly. Image analysis is included in the price.

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