UMKC Mass Spectrometry and Proteomics Core Services and Fees

Services: UMKC Faculty and CMH rates

protein identification-"in-gel"	**Includes**	**Fee**
Gel processing and data acquisition for Protein identification	In-gel trypsin digestion, extraction of peptides, and LC-tandem MS (data acquisition)	$175 for the 1st gel band, $120 for additional gel bands
Database search, Reporting	Mascot search	$10 per sample/data file
protein identification-"in-solution"	**Includes**	**Fee**
Sample processing of proteins submitted in solution	FASP (Filter-Aided Sample Preparation)	$250 per sample
Solid phase extraction	Sample clean-up, concentration	$25 per sample
TMT labeling (quant)	Isotope labeling for TMT quant	$100 per sample
Peptidome assay	peptide yield estimate-96-well format, with standard curve	$25 per sample
LC-MS only (no analysis)	Instrument use, nanoLC column	$100 (column), $25 per hour
Basic Reversed Phase offline fractionation	**Includes**	**Fee**
1st dimension for mudpit at high pH, orthogonal to acidic reversed phase (online LC-MS)	Micro-LC of peptidome (~20-25ug) and 96-well robotic fractionation (CTC-PAL)	$750 (same fee for between 6 to 24 fractions, as needed)
Second dimension (mudpit)	**Includes**	**Fee**
Acidic online reversed phase LC of fractions from 1st dimension	nanoLC-tandem MS data dependent acquisition	$120 per fraction
Database search, Reporting for protein identification only	Mascot search	$10 per fraction, pooled into a single search
Quantitative analysis (TMT) Database search, Reporting for protein identification and	Quantitative ratios, with GOterms, KEGG references, when available	$15 per sample, concatenated into a single search
Functional yield testing and balancing for quantitation	Yield assessments (by LCMS) for load balancing final samples	$50 per sample
Label Free quantitation requires at least triplicate runs for each sample/condition	Count on at least 3x LCMS runs per sample/condition, times each mudpit fraction, if mudpit is needed	$120 per replicate LCMS run, for each condition (ctl, expt, etc.) see example budgets below in section 3
Label Free Search search, and quantitative analysis, and Figures for publication	Quantitative ratios (raw and log(base2), GOterms, KEGG references, when available	$300 for each pairwise comparison
ESI-MS of relatively pure samples-when no LC is required	Nanospray analysis: sample in solution/dissolved in solution	$65 per sample

Note: External Academic/non-profit rates: add a 20% facility user fee to the above fees.

For a quote, or for a budget to include in a grant proposal or budgetary consideration, arrange for a meeting with the Facility director. There is no fee for consultation or for providing a budget. The above services are explained in more detail below the following table, which shows the fees for services offered for commercial/industrial purposes.

UMKC Mass Spectrometry and Proteomics Core Services and Fees

Services: External Industry rates:

protein identification-"in-gel"	**Includes**	**Fee**
Gel processing and data acquisition for Protein identification	In-gel trypsin digestion, extraction of peptides, and LC-tandem MS (data acquisition)	\$300 for the 1st gel band, \$150 for additional gel bands
Database search, Reporting	Mascot search	\$50 per sample/data file
protein identification-"in-solution"	**Includes**	**Fee**
Sample processing of proteins submitted in solution	FASP (Filter-Aided Sample Preparation)	\$500 per sample
Solid phase extraction	Sample clean-up, concentration	\$35 per sample
TMT labeling (quant)	Isotope labeling for TMT quant (user supplies TMT reagents)	\$35 per sample
Peptidome assay	peptide yield estimate-96-well format, with standard curve	\$50 per sample
Basic Reversed Phase offline fractionation	**Includes**	**Fee**
1st dimension for mudpit at high pH, orthogonal to acidic reversed phase (online LC-MS)	Micro-LC of peptidome (~20-25ug) and 96-well robotic fractionation (CTC-PAL)	\$1000 (same fee for between 6 to 24 fractions, as needed)
Second dimension (mudpit)	**Includes**	**Fee**
Acidic online reversed phase LC of fractions from 1st dimension	nanoLC-tandem MS data dependent acquisition	\$250 per fraction
Database search, Reporting (protein identification only)	Mascot search	\$25 per fraction, concatenated into a single search
Database search, Reporting for Quantitative analysis (TMT)	Mascot search	\$50 per sample, concatenated into a single search
ESI-MS of relatively pure samples-when no LC is required	Nanospray analysis: sample in solution/dissolved in solution	\$120 per sample

The details on the following pages provide more information about the above facility services. See other documents on the facility website for sample preparation methods and recommendations. Example budgets for LFQ analysis on page 3.

UMKC Mass Spectrometry and Proteomics Core Services and Fees

1. Protein identification (protein samples submitted in *polyacrylamide gels*)

Includes in-gel reduction/alkylation (cysteine modification), in-gel trypsin digestion, extraction of peptides, acidic reversed phase nano LC-MS (Data Dependent Acquisition using our Q-Exactive High Resolution MS system). Mass resolution will be set at 70,000 for MS scans, and 35,000 for dependent MS/MS scans in data dependent acquisition unless otherwise requested. This in-gel protein identification service includes gel documentation (your gel) with a high quality color scanner: the gel image will be provided with the protein identification report.

SDS-PAGE gel bands or gel swaths-rates for UMKC Faculty/CMH:
-$175 for the 1st sample, and $120 for each additional samples in a set.
The following prices apply to samples received from commercial/industrial sources:
-$300 for the 1st sample, and $150 for each additional samples in a set.

Database searches for protein identification with Mascot Server 2.5 or Proteome Discoverer utilize Swiss-Prot database, as the default database (updated at 6 month intervals), though another database can be searched upon request. The search includes decoy database (reversed) search for calculating False Discovery Rate (FDR). Refer to the tables for data search fees. Identified proteins will be provided as PDF documents from Mascot Search results. The individual PDF files show the identified protein and a summary of the peptide spectral matches (PSM) that support the identification. Gel documentation is also provided with the results as jpg image marked up with excised gel bands indicated. If you prefer to review your own search results, web links to online interactive Mascot search results for each sample are provided upon request at no cost for each sample, and there is a data search interpretation guide available on the Facility website at this address http://sbs.umkc.edu/research_proteomics.cfm.

***ESI-MS** (nanospray) service by direct electrospray infusion is offered for pure samples that have no salts or contaminants (ready to infuse- when no Reversed Phase LC required). This service assumes low complexity of samples (synthetic peptides, RPLC fractions, compatible purified organic compounds, etc): $65 per sample (Faculty/CMH), $120 per sample (industry).

2. Protein identification (protein *in-solution*), optional quantitation w/ Tandem Mass Tags

In-solution digestion is done using Filter Aided Sample Preparation (FASP) as an alternative for samples that are submitted in solution. FASP is accomplished with the use of microcon spin filters to retain the proteins during processing steps, which includes reduction, alkylation and trypsin digestion. The resulting peptides are harvested and purified for LC-MS. FASP is typically done in conjunction with samples of higher complexity (total cell lysate, serum, sub-cellular fractions, etc.) when 100s, or many 1000s of proteins are anticipated. Depending on the number of proteins expected, a single run (RP-nanoLC tandem MS) may be sufficient. However, offline (benchtop) basic reversed phase pre-fractionation may be necessary for more complex samples where multidimensional liquid chromatography is needed (mudpit-see next page). For comparative quantitation between samples, FASP can be combined with Tandem Mass Tag (TMT) labeling protocol. FASP sample preparation and optional TMT labeling, showing rates for UMKC Faculty/CMH:

-FASP (*see top of next page)	$250/sample
-Solid Phase Extraction (SPE)	$25/sample (recommended for TMT)
-Optional TMT labeling (quant)	$25/sample (user provides reagents)
-Protein/peptide assay	$25/sample (recommended for TMT)

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*What is compatible with FASP? Samples may contain urea, SDS, reductants, salts, moderate organic content (less than 20% acetonitrile, for instance). Ask about concentration/volume. FASP should be preceded by the protein/peptide assay, if needed, to ensure the processing of an optimal quantity of protein. The user may provide that information based on protein assay prior to submitting the sample. When TMT labeling is done on whole cell extract, 100ug of protein is recommended. The SPE step is required for sample cleanup whether or not TMT is done. When TMT labeling is done, protein assay is done (required) AFTER the labeled peptidome is purified by SPE because TMT samples must be mixed ~equimolar (at 1:1:1:1… ratio) for best results in quantitation (load balancing). We use either a 96-well Bradford assay, utilizing a standard curve generated from a tryptic digest of BSA (Albumin), or alternatively, functional LCMS test runs.
-The protocol for FASP is a variation on this adaptation of FASP for TMT labeling:
McDowell,G.S. Aleksandr Gaun, A. and Steen, H., iFASP: Combining Isobaric Mass Tagging with Filter-Aided Sample Preparation, *J. Proteome Res.*, 2013, 12 (8), pp 3809–3812

3. Label Free Quantitation (LFQ)- whole proteome differential expression analysis:

Label Free Quantitation analysis requires a separate sample for each condition (no multiplexing), **at least** in triplicate. Three biological replicates is recommended. Each pairwise comparison will need three LCMS runs for both conditions. Six LCMS runs is a minimum experiment, when no offline fractionation is needed (mudpit). If mudpit is needed, each pooled set of fractions from 1st dimension also needs to be run in triplicate. So, if 1st dimension generates 4 pooled samples, must be repeated three times for each condition (12 LCMS runs per condition). A pairwise comparison with 4 pooled fractions would therefore result in 24 LCMS datafiles. So as you can see, the inability to multiplex for LFQ substantially increases the number of LCMS runs needed.

Example budgets for LFQ pairwise analysis (two conditions) with 3 pooled fractions from 1st dimension (mudpit). Discounts apply since there is substantial duplication of processes:

FASP $250 per sample x 6 samples (2 conditions x 3 biological replicates) = $1500
1st dimension fractionation: 40% LFQ Discount
Yield testing (LC-MS runs for functional yield for LFQ balancing ($25/sample) = $150
1st dimension fractionation (Mudpit) $750 x 6 samples = $4500 - $1800 = $2700
LCMS- multiple replicate 25% LFQ Discount
LCMS, data acquisition 6 samples x 3 pooled fractions $120 x 18 = $2160 - $540 = $1620
LFQ search, and quantitative analysis, and Figures for publication $300
(subtract 4x FASP, 4x yield testing for 2 samples, not 6 (tech. replicates only). **Total: $6270**

If you would like to screen for differential expression without the biological replicates for a quick pairwise comparison with no mudpit, a lower cost option can be done. But for analysis, you still must have **at least 3** LCMS files each (technical replicates-that is 6 LCMS runs total). Here is a budget for such a screening experiment:

FASP	$250 per sample x 2 samples	= $500
SPE cleanup	$25/sample	= $50
Yield Testing, balancing for LFQ loading (LCMS testing)	$50x2	= $100
LCMS data acquisition	$120 x 6 runs	= $720
Pairwise analysis $300	db search/quantitation	= $300
		Total: $1670

For a sample with low complexity (bacterial proteome, for example), this pairwise screening (technical replicates only) is a viable alternative. Keep in mind that lower abundance proteins will not be identified, and if the sample is too complex (>2000 proteins or so), overlapping peaks will cause a problem (LFQ is based on MS peak intensity, so mudpit is then recommended. Mudpit can increase the number of identifications and improve quantitation of any proteome including bacterial proteome proteomes, but it is required for more complex samples to minimize overlapping peaks.

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4. Mudpit includes 1st dimension LC fractionation:
Basic reversed phase (BRP) pre-fractionation of the peptidome prepared by FASP is done to distribute the complexity into fractions prior to online acidic nano LC-tandem MS. Mudpit may be done when only protein identification is needed (no TMT quant), but is typically done when TMT quantitation is the goal. The extent of fractionation may be modest (say, 4 to 6 fractions), depending on the number of proteins present in the sample. However, it is recommended that for total cell lysate, a minimum of 12 BRP fractions are taken. More may be desired to dig deeper into the sample to identify and quantitate additional less abundant proteins. The nanobore micro LC columns for this first dimension are custom packed onsite (0.4mm I.D.), and fractions are taken with a CTC-PAL robot. These fractions are dried down in preparation for nanoLC-MS online with the Mass Spectrometer. The fee for pre-fractionation is the same, regardless of how many fractions are taken. One pre-fractionation run is done for each unlabeled sample, or for each combined TMT-labeled sample set.

Basic Reversed Phase 1st dimension Fractionation $750 per sample (UMKC Faculty/CMH)

5. Online Acidic Reversed Phase nanoLC Tandem HRMS data acquisition
Each sample (fraction) is subjected to nano LC-Tandem MS under typical acidic reversed phase conditions online with the High Resolution Mass Spectrometer (Q-Exactive Plus) for data acquisition. Custom hand packed columns (reversed phase matrix) are prepared onsite and tested with a standard digest sample prior to going into service. A standard 90 minute gradient and data dependent acquisition is typically used for these analyses, generating over 20,000 tandem MS scans per fraction/run for complex samples at resolution 70,000 (MS scans) and 35,000 (MS/MS) for quantitative TMT analysis. Resolution can be tailored to the needs of the experiment (up to 120,000). However, we may find that lower resolution in the MS/MS scans is possible to increase the number of proteins identified/quantitated. Faster scan modes may be utilized for "identification only" experiments to dig deeper at lower mass resolution.

90-min acquisition (acidic RPLC-tandem MS) $120 per sample/fraction (UMKC Faculty/CMH)

6. Database searches are conducted on your choice of database (UNIProt/SWISProt is the default database), including a decoy database for calculating False Discovery Rate (FDR). Database Searches for LC-MS data files for mudpit experiments using Proteome Discoverer and Mascot protein identification programs are conducted by concatenating the files into one search, with fee based on the number of fractions: for UMKC Faculty and CMH- $10 per fraction for identifications, and $15 per fraction for TMT quantitative identification analysis (to a maximum of $100 each set of fractions). Identifications can be provided in a spreadsheet with selected information columns (Accessions, protein name, scores, % coverage, quantitative ratios, etc). A web link to search results is provided upon request for interactive search results browsing. Protein quantitation information is included in the spreadsheet when applicable, where the differential expression between controls and samples are provided as a ratio, based on planning with the investigator. A guide for quantitative interpretation (and qualitative-identifications) is available on the facility website to refer to while browsing the results at your desktop, and assistance and training in search results interpretation can also be provided by appointment.

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All sample records, and all sample data is permanently stored in the core facility or with a secure cloud service. These records can be transferred to your media storage devices or cloud service if you would like to duplicate the records. Assistance with methods/procedures for manuscripts, Figures, Figure legends and Tables are provided with identification/quantitation services at no additional cost.

Database searches for **Label Free Quantitation (LFQ)** are done for each pairwise comparison ($300 per quantitative pairwise comparison), independent of how many fractions may or may not have been taken (optional mudpit). This includes assistance with the preparation of Figures, and methods description for publication. Also, your data files and anything required by a Journal for publication will be uploaded when you submit a manuscript. SwissProt database is the default database for searches. Alternatively, you can specify the database that you want to include, such as a bacterial proteome that you have, or newly sequenced species that has a hypothetical translated proteome available at UniProt or NCBI which is not represented in SwissProt.