User Submission Guidelines

Sample Requirements

These requirements will ensure efficient handling of samples within the core and allow the team to provide results in a timely manner. Thank you!

Experimental Design

For pilot experiments, single replicates can be run to assess potential differences.

For experiments that will be used for publication, 3+ biological replicates are required. 6 biological replicates are encouraged for the benefit of increased statistical power during subsequent analysis.

Sample Amount

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Amount/Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Samples</td>
<td>3-5 mg dry weight (15-20mg wet weight) is sufficient, but larger samples are preferred if available</td>
</tr>
<tr>
<td>Cell Cultures</td>
<td>1-2 million cells per sample is ideal</td>
</tr>
<tr>
<td>Biofluids (e.g. plasma, RBCs, urine)</td>
<td>A minimum 50-100 uL is required</td>
</tr>
<tr>
<td>Pulldown Assays (e.g. Co-IP)</td>
<td>25-50µg protein per sample</td>
</tr>
</tbody>
</table>

Sample Preparation

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Samples</td>
<td>Freeze in pure/untreated form</td>
</tr>
<tr>
<td>Cell Cultures</td>
<td>Wash 3x with cold PBS, and freeze cell pellets in 1.5-2 mL Eppendorf MS-compatible microcentrifuge tubes devoid of all supernatant.</td>
</tr>
<tr>
<td>Biofluids (e.g. plasma, RBCs, urine)</td>
<td>Freeze in pure/untreated form</td>
</tr>
<tr>
<td>Pulldown Assays (e.g. Co-IP)</td>
<td>We recommend secondary validation of potential binders prior to MS/MS analysis. Include a non-specific control sample</td>
</tr>
</tbody>
</table>

ALL samples must be free of salts (including non-volatile buffer salts) and non-volatile solvents, for example, DMSO, DMF, and glycerol.
Minimize the Contaminants

While the team strives hard to obtain the best results possible, the quality of the data generated by the mass spectrometers within the facility is only as good as the quality of the samples introduced into them. Hence it is imperative that the samples intended for MS analysis be prepared by taking extreme care to avoid contamination.

- Use freshly prepared solutions/buffers when preparing samples for proteomics analysis.
- When utilizing strong acids use glass pipettes to avoid any polymer degradation and accumulation within samples of interest.

Sample Submission Process

Submission in Person

1. Contact the core at SOMproteomics@cuanschutz.edu to schedule sample drop-off.
2. Complete submission form.
3. Please provide samples frozen in 0.6-1.7 mL labeled microcentrifuge tubes.
4. Samples must be in a standard size freezer box labeled with submitter name, PI name, submission date, and project identifier from sample confirmation.
5. Bring samples on dry ice to the Proteomics Core (RC1-S, first floor, room 1303). If you do not have RC1-S access, please contact core personnel for assistance.

Submission by Mail

1. Contact the core at SOMproteomics@cuanschutz.edu to schedule delivery.
2. Complete submission form.
3. Please provide samples frozen in 1.5-1.7 mL labeled microcentrifuge tubes.
4. Please package on dry ice and include a hard copy of the sample list that is labeled with submitter name, PI name, and project identifier from sample confirmation.
5. Please overnight on Monday or Tuesday to the below address. If shipping from outside North America, please ship on Friday or Monday to the following address:

   SOM Proteomics Core
   University of Colorado Anschutz Medical Campus
   12801 E 17th Ave, Room 1303
   Aurora, CO 80045 USA
What to Expect (Deliverables)

After analysis, you will receive all data in Excel spreadsheet format (.xlsx). This tabular data will contain protein and peptide identifications reported as both peptide spectral matches (PSMs) and area under the curve (AUC) intensity. Data can also be provided in Scaffold (Proteome Software) by request.

Additional data analysis, including figure generation and statistical analyses, can be performed for an additional cost of $120 per hour of analysis. Bioinformatics can also be performed through the Biostatistics and Bioinformatics Shared Resource (BBSR).

Notes on Authorship

Co-authorship is generally expected when core personnel have made significant contributions to the research in the form of consultation, experimental design, method development, data analysis and/or data interpretation.

Significant is defined as "the project would not have progressed, or progressed at a substantially slower pace, without the guidance of facility personnel." In such cases, core personnel should have the opportunity to review and edit the appropriate sections of a manuscript before submission.

When the conditions of co-authorship are not met, acknowledgement of facility contributions is expected in publications that include data generated in the facility or processed using core instrumentation or software. An example of appropriate acknowledgement is "The authors wish to thank the University of Colorado School of Medicine Biological Mass Spectrometry Proteomics Core Facility for their contributions to this manuscript."

Please inform us when relevant publications are accepted, as we post citations on our website and in our annual reports. This information is vital to the continued support of the facility.