How can we submit our samples to MSPCF?

Please contact any of us by either email or phone and we will be happy to help. Also, before you send your samples to us, please fill out or Submission form.

What buffer should my sample be in before I submit to MSPCF?

We prefer samples to be submitted as Coomassie-stained SDS-PAGE gel cuts. Gels should be thoroughly de-stained in water and delivered in a clean & keratin-free container. Samples can also be submitted as cell/tissue lysates and will be run on our pre-cast gels ($16 extra charge/gel). Please don't mix your sample with Laemmle sample buffer, as it is not compatible with our gels. Sample volume should not exceed 50 µL. On request and depending on the project needs, we can also perform in-solution digestion.

Are detergents in my sample a problem? How can I remove them?

Most detergents (SDS, LDS, Tween, Triton, NP-40, Octyl glucoside, CHAPS, etc.) are a big NO for mass spectrometry analysis and should not be contained in your samples. If they cannot be omitted during sample preparation, they MUST be removed. For this, please use "Wessel-Fluegge protocol". Alternatively, you can use LC-MS compatible detergent, such as RapiGest SF, PPS silent Surfactant, Invitrosol, ProteaseMax, as they degrade during proteolysis or do not overlap with the proteolytic peptides elution profiles.

How much protein is necessary for identification or quantification?

In general, Coomassie stained gel bands have a high success rate for identification. We recommend loading up to 50 µg of proteins per well for a complex sample mixture and a minimum of 50 ng of a single protein band.

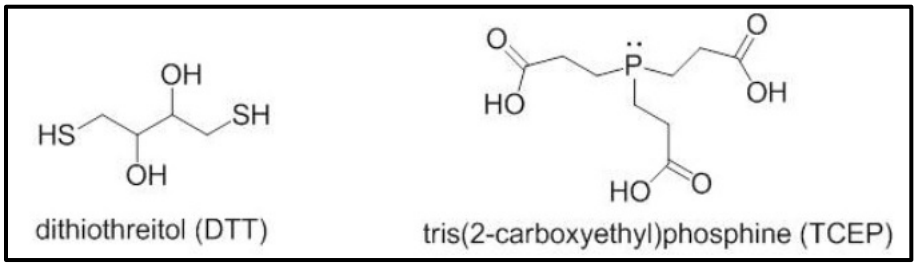
Can I submit silver stained gels?

Please don't. We rather recommend using Coomassie blue dye. Alternatively, you can use the new developed mass spectrometry compatible silver stain, such as one from ThermoFisher Scientific

Reduction: TCEP or DTT?

DTT is a thiol containing reagent. It should not be used in experiments involving thiol labeling or isoelectric focusing. Aqueous solution of DTT is quite acidic.

TCEP HCl is odorless, air-stable and reduces disulfides at room temperature in less than 5 min. It is reactive at a broad pH range. TCEP is a great reducing reagent because it does not consume iodoacetamide during alkylation step, unlike -SH containing DTT. Never store TCEP in phosphate buffers, as it will decompose.



Ambic or TEAB?

Ammonium bicarbonate (ambic) is a volatile salt, which breaks down to NH_3, CO_2 and H_2O. Volatile salts are the only salts compatible with mass spectrometry. Aqueous solutions of ambic have pH around 8.0, the optimal pH for trypsin activity. Ambic competes with basic amino acids for Coomassie dye, which makes it a great de-staining reagent for the in-gel digestion method.
Another ammonium salt, triethylammonium bicarbonate (TEAB), is more volatile than ambic. It is also more expensive. Choose TEAB over ambic for your TMT or iTRAQ experiment.

How can we do a peptide quantification?

There are several options for this. Possibilities include label free quantification, chemical isobaric peptide tagging (TMT or iTRAQ) or metabolic labeling (SILAC). While we can help you with quantification using isobaric tagging kits or label free, costumers are responsible for their SILAC experiments. SILAC media can be obtained from various suppliers.

Can you help us detect protein PTMs?

Yes! Please mark this option when filling out our Submission form and we will discuss it with you. Mass spectrometry is a powerful tool, and can detect various protein PTMs.

When can I expect my results?

We process the samples following a "first come first serve" idea. Processing time might vary, depending on the total sample number in the queue as well as the complexity of the workflow. An individual approximation of the processing time will be given during sample

submission. We try our best to deliver MS data back to our customers as soon as possible.