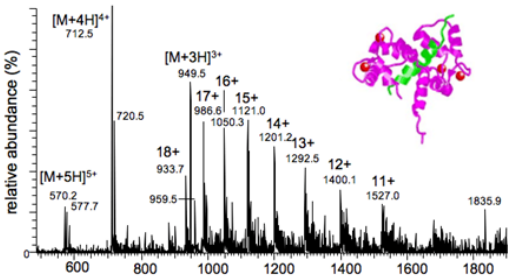
DBMR, Proteomics & Mass Spectrometry Core Facility

UNIVERSITÄT BERN



Guidelines for Sample Submission:

General Info

1. Please contact us about the details of your sample and submit your sample together with our sample submission form (page 2 of this document).
2. For gel samples, we recommend any Coomassie or fluorescence dye **over** silver staining. In case you insist on silver staining, please make sure that a mass spectrometry compatible silver staining protocol or kit is.
3. For in-solution samples, we request from you an accurate estimate of protein concentration and buffer composition. Our preferred buffer solution is 8M urea in 100mM TRIS/HCl pH 8.
4. The more information you provide to us about your sample, the better we can handle your sample and apply the most appropriate analytical procedure.
5. We do our best to process your sample as soon as possible.
6. Identification results are generally provided with a 1% false discovery rate on the peptide level in form of an excel file, downloadable from the UniBe ILIAS website.

Background Information

The detection limit of proteins from SDS-PAGE gel bands with the use of LC-MS/MS is in the femto-mole range, corresponding to the low nanogram range (1-5 ng). This is also very similar to the detection limit of proteins stained with Silver and fluorescence dyes. Colloidal Coomassie stains have detection limits between 10 and 20 ng and the common Coomassie Brilliant Blue is around 50 ng. The detection limits of these stains will be dependent on many variables such as the protein itself, the thickness of the gel, and the width of the lane. The detection limit of protein staining is governed by protein mass, whereas the detection limit of mass spectrometry is by molarity, hence larger proteins have higher detection limits for the mass spectrometer. (For instance 1 ng of a 20 kDa protein corresponds to 50 fmol, while a MW of 200 kDa corresponds to only 5 fmol! Both proteins will have similar staining intensities on the gel, but there is 10 times less protein on a molar basis from the 200 kDa protein.) Furthermore, be aware that the in gel digestion procedure has rather low peptide yields of 10 to 30%!!!

Avoid Keratin Contamination

Keratin is the most commonly observed background contamination for samples analyzed by Mass spectrometry. Keratins are part of dust particles!

Work always with gloves, on dust-free surfaces; wash all surfaces and tools with 70% ethanol!!!

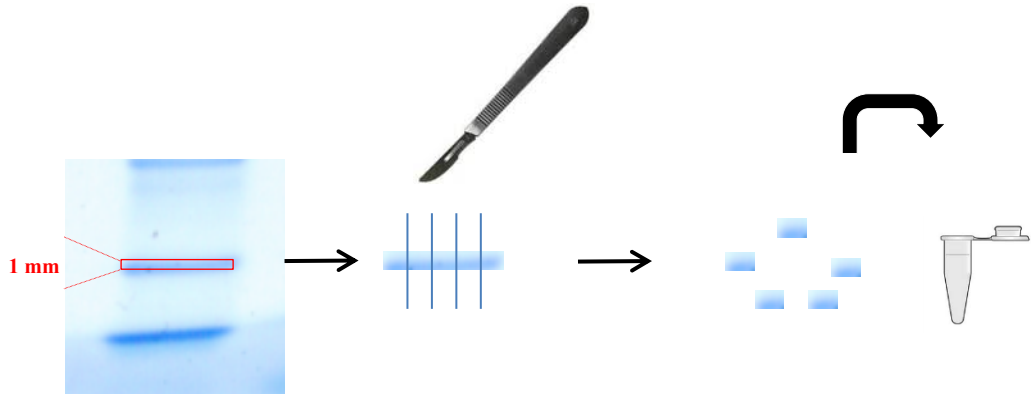
Preparation of samples for in-gel digestion/LC-MS^2 of bands from SDS-PAGE and on-membrane digestion/LC-MS^2 of proteins blotted onto PVDF

1. For Coomassie staining: De-staining gel to a clear background can be an advantage.

   For Silver staining: Stain only as long until bands of interest appear (usually only a few minutes); avoid over-staining!

2. If possible, take a picture of the gel prior to excision of gel bands and submit an image along with the sample.
3. Cut gel bands with scalpel accurately along stained region. Please cut only a vertical segment in the middle of the band in case of a fat and broad gel band.
4. Please avoid any gel pieces from the stacking/separation gel boundary. **Please note that low % acrylamide gel pieces (stacking gel) do block our LC setup!**
5. Slice bands into small (~1 mm^3) cubes.

Do not deliver more than 6 small cubes of ~1 mm^3.



6. Transfer gel cubes into a clean, keratin-free 1.5 ml-reaction vial with lid (Eppendorf type). **Please NO tubes with screw caps!**
7. In case you provide Silver stained SDS-PAGE samples, we request you to destain the gel pieces according to our recommended protocol found under Analytical Services.)
8. Cover gel cubes with liquid composed of ethanol/water 20:80.
   Pieces of membranes are kept dry.
   Under this condition, samples can be stored for several weeks at -20° or 4°C.
9. Label tubes with permanent ink with your name and the sample identifier.
   Please do not label the lid!
10. Keep samples at 4°C and bring them to us at Murtenstrasse 28 with the filled-out sample submission form:

Laboratory PMSCF, 4th floor, lab 474

Thank you!