

PROTEOMICS PLATFORM

McGill University and G enome Qu ebec
Innovation Centre

Proteomics Services Sample Preparation User Guide

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General Guidelines for Sample Preparation

When working with biological samples that are destined to be submitted to the Proteomics Platform, to avoid contamination of your samples with human keratin shed from your skin, hair and nails, we recommend during handling that you:

- Wear new gloves
- Wear a clean lab coat
- Wear disposable sleeves
- Wear a hair net
- Wear a mask
- Avoid excessive handling of your samples.
- Avoid bending over your gel.

There is a direct correlation between the quality of results obtained and the quality of sample preparation.

Preparing samples for 1D gel fractionation

Samples submitted for 1D gel electrophoresis can be submitted in Laemmli buffer. If not, please provide us with the exact buffer composition.

Please also provide us with the amounts of proteins present in each sample.

For the analysis of complex biological samples we recommend 30-50 μg of total proteins per sample.

The maximum volume for fractionation on 1D pre-cast gels is 15 μl and 45 μl onto 1D mini-special gels. Protein samples in higher volumes will have to be precipitated with TCA or acetone prior to being loaded onto gels.

Preparing samples for 2D gel fractionation

Samples submitted for 2D gel electrophoresis will be precipitated and quantified in house prior to fractionation to minimize distortion due to the presence of detergents or other reagents that could affect migration.

A minimum of 50 μg of total protein per biological sample is required.

Preparing samples for band/spot excision

You can submit to the Platform your own gel and we will manually or robotically excise bands or spots that are of interest to you.

We recommend that each excised band/spot contains at least 1 pmole of proteins (e.g. 50 ng of a 50 kDa protein). Lower amounts can result in poor proteomics results.

We recommend that proteins are stained with Coomassie Blue since silver staining has a detrimental effect on the activity of trypsin, the enzyme used to digest proteins into peptides prior to mass spectrometry.

If proteins on your gel have been stained with silver, protocols used for the staining must be compatible with mass spectrometry. The protocol that we recommend is available upon request.

If you have to use silver staining, we don't recommend that the development lasts more than 2 minutes. Developing longer will result in poor proteomics results.

Please note that silver staining is a very sensitive method which can rarely provide reliable estimates on the amounts of proteins based on intensity of staining.

Excising samples for in gel digestion

You can submit to the Platform bands or spots that you have manually excised.

If you excise your own bands/spots, make sure to clean up the support onto which the gel will be put.

Cut as precisely as possible bands/spots of interest. Excess polyacrylamide is superfluous.

1D gel bands can be manually excised with scalpels.

The maximum size of a band should not exceed 20 mm² in size (e.g. 1 cm wide per 2 mm high).

We recommend that each excised band/spot contains at least 1 pmole of proteins (e.g. 50 ng of a 50 kDa protein). Lower amounts can result in poor proteomics results.

Put each excised band in an Eppendorf tube and add 1% acetic acid or de-staining solution.

We recommend that proteins are stained with Coomassie Blue since silver staining has a detrimental effect on the activity of trypsin, the enzyme used to digest proteins into peptides prior to mass spectrometry.

If proteins on your gel have been stained with silver, protocols used for the staining must be compatible with mass spectrometry. The protocol that we recommend is available upon request.

If you have to use silver staining, we don't recommend that the development lasts more than 2 minutes. Developing longer will result in poor proteomics results.

Please note that silver staining is a very sensitive method which can rarely provide reliable estimates on the amounts of proteins based on intensity of staining.

We strongly suggest that users send us a scanned image of their gel. The size of the image must be close to real size of the gel.

Preparing samples for in solution digestion

Samples can be prepared for in solution digestion.

We recommended to send samples in 50mM ammonium bicarbonate pH 7.8/6M urea in the smallest volume as possible (e.g. 10 ul).

If you can't put your samples in the buffer suggested above, please inform us of the composition of the buffer the samples are in.

We recommend that 15-75 fmole of each protein.

Given that detergents and other reagents can impair detection of peptides at the mass spectrometry level and/or can damage the analytical columns, please note that your samples will be subjected to a cleanup procedure in order to avoid problems at the LC-MS analysis stage.

We recommend that you analyze on 1D gel an aliquot of the sample destined to be digested in solution to get an idea on its content prior to submission to the Platform. In case of poor results, the protein profile can provide useful hints as to reasons why mass spectrometry has failed.

Submitting Samples to the Platform

General considerations

- Please fill out the latest version the [Request Form](#) that is available on the web site.
- Send it electronically to infoservices@genomequebec.com
- Print the first page of the Request Form. It must be signed by the authorized person and sent alongside biological samples.
- A Purchase Order or an indication of payment by credit card must be provided. Your number will be asked when you will receive your invoice
- If your samples are from human subjects, please also include a copy of the appropriate ethics review approval forms.

Please note that omitting one of the steps mentioned above might result in delays in processing your samples.

Preparing samples for shipment

- Once your samples are placed in Eppendorf tubes, we recommend putting parafilm around the top to prevent leaking during transportation.
- If you send samples by mail, please indicate 7104 as the room number for delivery.
- If you bring yourself your samples, please take an appointment by emailing to the Customer Manager Office infoservices@genomequebec.com prior to your visit.
- Gels or gel bands/spots should be sent at room temperature or at 4°C (never freeze acrylamide)
- Protein samples in sample buffer (such as Laemmli, LDS, etc.) can be sent at room temperature, at 4°C or on dry ice. We recommend avoiding multiple freezing/thawing cycles.
- Precipitated proteins (e.g. acetone precipitated sample) should be sent at temperatures below 0°C.
- Sample in other buffers such as water, PBS, etc must be sent at 4°C or on dry ice.
- Proteases can be active at temperatures > 10°C.

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