

BSRC “ALEXANDER FLEMING” PROTEOMICS FACILITY

GUIDELINES AND RECOMMENDATIONS v.2

Please try to make sure that the samples you submit follow these guidelines as closely as possible. Keep in mind that the pre-analytical procedures, i.e. sample collection, preparation (if applicable), storage and shipping, affect the quality of your results and are as important as the analytical steps undertaken at our Facility. If in doubt about any of these guidelines please contact us. We will be happy to discuss them with you and clarify any questions you may have.

PROCEDURE TO FOLLOW FOR YOUR PROTEOMICS EXPERIMENT

1. Completion and submission of the “General Request Form”.

The Proteomics Facility will contact you within 5 working days of receiving your completed form to discuss your project in more detail, to advise you on the preparation, storage and shipping of your samples, and to clarify any questions you may have. An in-person meeting or a zoom call can be arranged if a more extended discussion is required.

2. Sample preparation.

The facility accepts many different types of samples for proteomics analyses (e.g. gel bands, immunoprecipitations, cell pellets, animal tissues, plant tissues, biofluids, etc). The amount of sample required as well as specific guidelines for your project need to be discussed with us before starting your experiment.

Some general guidelines:

- Avoiding contamination: Try to work in a clean environment as much as possible. Spray your working area with 70% ethanol. Wear gloves (nitrile if possible, not latex) and a lab coat to avoid keratin contamination. Use good quality plastic tubes (e.g Eppendorf or Sarstedt) and use clean glassware (avoid washing glassware with detergents). The presence of compounds leaching from bad quality plastics as well as the presence of detergents interfere with mass spectrometry analyses.
- Tubes used: Put your samples in low-binding polypropylene tubes if possible.
- Tubes labeling: Make sure samples are labelled clearly with a permanent pen and are easy to read. Do not wrap in plastic or stick sellotape on tubes.
- Tissue culture samples: Wash/remove all FBS and BSA traces.

- Blood samples: Try to assure a good practice for blood collection from your collaborators. Make sure samples are not hemolyzed or highly lipemic.
- All clinical samples: see Legal Restrictions at the end
- Replicates and samples for comparison: Make sure you have included all biological replicates required for your analysis as well as all of the appropriate controls. Please note that all samples need to be submitted at the same time for quality control purposes. If your experiment requires the preparation of multiple sets of samples (e.g. patient material that needs to be collected at different time points) you are required to finish all of your sample collections/preparations before submitting your samples to us. The Facility cannot store samples while you gradually accumulate your data sets (in the case where your data sets span years, the setup of the project needs to be discussed with us)
- User: Ideally sample preparation must be carried out by the same user and on the same day or days for all of the samples (if possible). Simultaneous processing of samples ensures conditions are identical among the samples, which is fundamental for their downstream analysis. Depending on your project and the nature of your particular samples, decide which preparation and storage conditions will give you the most reliable and reproducible workflow.

3. Completion and submission of the Sample Submission Excel.

Once your samples are prepared and ready for shipping, download and fill out the "Sample Submission Excel". Fill out only columns A to M (do not write in columns N to Y). Label the file with your full name and the date of submission. It is important that the excel file is properly filled out and that no information is missing.

Send your completed excel file to proteomics@fleming.gr together with any additional files, if you think they are necessary (could be text documents or images). Please do not send or bring your samples to the Facility without having submitted a completed excel file.

4. Shipping samples.

Please use the proper packaging for your samples. Make sure vials are tightly closed and properly labelled. Make sure that your samples are securely placed in a clean bag/container, keeping in mind that prior to processing by the Proteomics Facility they may need to be handled by non-laboratory personnel upon receipt.

Be advised that it is the responsibility of the user to ascertain whether the samples contain any toxic chemicals or infectious agents prior to their shipping.

Please do not send any loose papers together with your samples. All relevant information must be included in the "Sample Submission Excel", to be filled out and emailed to us at the time of shipping.

ADDITIONAL INFORMATION

Sample storage

Please note that any surplus material of your samples will be stored in our facility for a maximum of 6 months after the completion of your proteomic experiment. After that date, samples not collected by the user will be discarded.

Data analysis, transfer and storage

Please let us know when submitting your samples whether you will need any data analysis. Analysis can vary depending on each individual project, but in general will include library searches, statistical analysis and basic data visualization. For data analysis comparisons, please indicate in the submitted excel what is the group for each of your samples. Suggestions for analysis software and relevant tutorials can also be provided by us for further analysis by the user.

Following the completion of your experiment and its corresponding data analysis, the transfer of your raw and analysed files will be done after consultation with us. Please note that due to space limitations your data can only be stored in our facility for a maximum of 15 days after which time we will have to delete it. Proteomics data take up extensive storage space so you are strongly advised to be equipped with an external storage device of adequate space for all your proteomic experiments.

Legal restrictions

Please note that due to GDPR restrictions, human samples labelled with donor/patient names will not be accepted by the facility. It is the user's responsibility to transfer the samples to new vials labeled with a different system prior to shipping. Also note that for human samples all volunteer/donor/patient/etc consent procedures and forms are the user's responsibility. Samples received and analysed by the BSRC Fleming Proteomics Facility will be assumed to comply with all of the necessary legal requirements.

Final note

Last, but not least, we would like to point out the importance of a well thought out research plan for your proteomics experiments, including the adherence to the above guidelines, which will help us give you the best proteomics results possible.

For queries, please call the BSRC Proteomics Facility on 210-9656310 ext. 152 or send an email to proteomics@fleming.gr

We look forward to working together!