GUIDELINES AND INFORMATION FOR CUSTOMERS OF CFMP ZMBH

Proteome analysis: Identification and quantification of proteins from cellular extracts or tissue

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1. Short description

Protein quantification in mass spectrometry involves determining the absolute or relative abundance of proteins within a sample. This is crucial for understanding cellular processes, comparing biological conditions, and identifying proteins associated with specific functions or diseases. The quantification process utilizes various strategies, including label-free approaches or stable isotope labeling, combined with mass spectrometric analysis to provide insights into the dynamic changes of the proteome.

Your samples will be processed using SP3 method [ref], which is able to remove detergents present in the sample prior to digestion and measurement by LC-MS/MS.

To limit polymer contamination, avoid autoclaving tubes and pipet tips used for the proteomics experiment.

2. During initial meeting inform us about:

- Do you already have an iLab account? [link]
- Brief overview about biological background, cell types used and biological question.
- Which protocol is used for protein extraction? Please provide a detailed protocol.
- Did you perform a test experiment? Can candidate proteins be monitored by Western blot? Did you check for reproducibility of sample preparation by SDS-PAGE/Coomassie staining?
- Did you consider stable isotope labeling (SILAC, dimethyl labeling, TMT)?

3. Sample preparation

Below, we provide two protocols suitable for efficient cell lysis of human cells. The protocol should be adjusted accordingly by your research group. Please, be aware that several factors may affect efficiency of protein extraction (e.g. growth phase of microorganisms).

Cell lysis Protocol A is beneficial for extraction of membrane proteins, whereas Cell lysis Protocol B avoid use of detergents, which ease the sample handling.

Cell lysis (Protocol A):

Note: All reagents, solutions and vessels should be of high purity and keratin free to obtain optimal results. Please, keep in mind that provided here protocol should be tested before performing the final experiment!

Materials:

RIPA lysis buffer (Sigma R0278)

Reagent	Final concentration
Tris-HCl pH 8.0	50 mM
NaCl	150 mM
Triton X-100	1% (v/v)
Sodium deoxycholate	0.5 % (w/v)
SDS	0.1 % (w/v)

Note: Add protease and/or phosphatase inhibitors prior to use. One milliliter of buffer should be sufficient to lyse approximately 5 million cells.

Benzonase (Merck 70746-3)

Reagent	Final concentration
Benzonase (>90%, 25-29 U/µl Merck, Darmstadt, Germany)	300 U/ml

Example of protease inhibitor mix: cOmplete Tablets Mini EDTA-free (Roche 4693159001)

Procedure:

1. Perform procedure on ice or in the cold room.
2. Wash cell pellets twice (usually done with PBS).
3. Lyse cell pellets using 100-200 µl RIPA buffer supplemented with 1% protease inhibitor mix (one well from 6-well plate).
   Note: add phosphatase inhibitors if needed

4. Incubate the cell suspension on ice for 5 min before adding 300 U/mL of benzonase to degrade DNA.
5. After additional 10 min incubation on ice, centrifuge the cell suspension at 18,000 g for 45 min at 4 °C.
6. Resulting supernatant can be snap-frozen in liquid nitrogen and stored at −80 °C. Protein concentration should be determined at this point. **DC assay is recommended (Bio-Rad 5000112).**

PAUSE POINT

Cell lysis (Protocol B)

Materials:

Note: All reagents, solutions and vessels should be of high purity and keratin free to obtain optimal results. Please, keep in mind that provided here protocol should be tested before performing the final experiment!

- Urea lysis buffer
  6 M Urea (MW 60.06 → 3.6g/10ml)

  100 mM NaCl (MW 58.44 → 0.058g/10ml)

  50 mM TEAB, pH 8.5

- Protease inhibitor tablets
  Complete Mini EDTA-free Protease Inhibitor tablets (Roche, 1 tablet for 10 ml lysis buffer)

- Optional: Phosphatase Inhibitors
  PhosSTOP Phosphatase Inhibitor tablets (Roche, 1 tablet for 10 ml lysis buffer)

- Benzonase 10ku: 70746-3 from Merck/Sigma

Procedure:

1. Prepare lysis buffer by adding protease inhibitors and optionally phosphatase inhibitors.
2. Wash cell pellets twice (usually done with PBS).
3. Add appropriate amount of lysis buffer (we use 1,5ml for a 150cm^2 plate) to the cells, resuspend by pipetting and incubate on ice for 10 min.
4. Cells were harvested using cells scraper and transferred to chilled 2ml Eppendorf tubes. Each sample was treated with Benzonase (final concentration: 0,25 U/µl -> 1:100 from the 25 U µL^{-1} stock) for 10 min. on ice.
5. Centrifuge 45 min at 4°C at 18000g to remove cell debris. → Transfer supernatant to fresh tube
6. Freeze in 200µl aliquots in liquid N_2. Keep aliquots at -80°C.
7. Determine protein concentration using **Bradford assay (Bio-Rad #5000006).**

PAUSE POINT

Protein concentration determination

- We strongly recommend following the recommendations of the Bio-Rad manuals. In our hands, Bradford assay works well with BSA standard resuspended in water solution. BSA

standard for the DC assay should be on the other hand resuspended in RIPA buffer. BSA standard can be stored at 4 °C for few weeks (please, control for contaminants).

- We suggest preparing two different dilutions of your samples and perform the measurement in duplicate. If you obtain reproducible results, then we suggest to calculate the average protein concentration of your sample.
- It is highly recommended to analyze 20 µg protein of each sample by SDS PAGE/Coomassie staining to check the reproducibility of sample preparation. The better the quality of protein concentration measurement, the better will be the quality of the LC-MS measurement!

Sample aliquoting for LC-MS analysis

Label free quantification, SILAC and dimethyl labeling

- Prepare 10 µg of proteins per sample in 100 µl of appropriate buffer (e.g. **50 mM Tris pH 8.0** if cells were lysed with **RIPA buffer** or **50 mM TEAB pH 8.5** if cells were lysed with **Urea lysis buffer**)

4. General information

- We will provide you the results within 4 weeks from the sample submission
- For proteomics experiments, we recommend using at least 4 replicates per condition (e.g. 4x treated and 4x control). To achieve higher data quality, we recommend using 5 replicates. It facilitates the data analysis and interpretation of the results.