GUIDELINES AND INFORMATION FOR CUSTOMERS OF CFMP ZMBH
Title: Identification of protein interaction partners using proximity labeling (BioID) followed by in-gel digestion

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1. PURPOSE

This document describes the recommendations regarding sample preparation and submission to Core Facility for Mass Spectrometry and Proteomics for **identification of interaction partners using proximity labeling (BioID) followed by in-gel digestion.**

2. DURING INITIAL MEETING INFORM US ABOUT

- Do you already have iLab account? https://hmls.corefacilities.org/service_center/show_external/3564?name=core-facility-for-mass-spectrometry-proteomics
- Do you work with membrane protein?
- What is your control sample?
- Are you planning to use a detergent during cell lysis, washing or elution of the proteins?
- Did you already perform a test experiment using Western blotting or SDS PAGE/Coomassie staining as read out?

3. PROTEIN ELUTION FROM THE BEADS

Interaction between streptavidin and biotin is one of the strongest non-covalent interactions known in nature. Therefore, efficient elution of proteins from the beads is challenging. At the moment, we recommend releasing the proteins from the beads by boiling the beads in presence of SDS sample buffer. We recommend using **1x SDS sample buffer**. You can use commercial sample buffer or homemade. Here is the recipe for **4x SDS sample buffer**:

Component	Volume
Tris (1M, pH 6.8)	10 mL
SDS	4 g
B-mercaptoethanol	10 mL
Glycerol	20 mL
Bromophenol blue	0.1 g
dH_2O	To 50 mL

Elution procedure:

Mix beads with up to 40 µl of 1x SDS sample buffer. Incubate 10 minutes at 95 °C with shaking. Centrifuge briefly to collect the beads at the bottom of the tube. Transfer the supernatant to the fresh tube.

4. RECOMMENDATIONS

- If needed, use only detergents compatible with in-gel digest:
  SDS < 2%
  CHAPS < 4%
  NP-40 < 1%
- You can obtain an aliquot of fixing solution and colloidal Coomassie if you would like to run the gel in your lab but **you can run gel in our facility**. We offer commercial gels (20€ / gel). One can load protein marker and up to **9 samples**. The maximum sample volume is **40 µl.**
- In order to run the gel in our facility, please contact **Sabine Merker by e-mail** and set an appointment**.**
- Always, bring with you **your elution buffer. We strongly recommend** to load **neighboring lanes** with your **elution buffer.**
- To ensure proper separation on the gel, mix your **marker protein** with appropriate amount of **SDS sample buffer.**
- Use **commercial gels** (higher reproducibility and resolution, reduces contamination)
- Run your gel for **1 cm** (measure the distance from the bottom of the well to the running front). Then simply stop the electrophoresis, rinse the gel shortly with dH_2O, incubate with fixation solution for 20 min with moderate shaking and stain with colloidal Coomassie for 1-4h.
- Make a picture of the gel, **mark the lanes on the picture** (preferentially in PowerPoint) and upload it to iLab. File upload is possible after the project request is created.

5. GENERAL INFORMATION

- We will provide you the results within 4 weeks from the sample submission
- For BioID experiments, we recommend using at least 3 replicates per condition (e.g. 3 x bait and 3x control). To achieve higher data quality, we recommend using 4-5 replicates. It facilitates the data analysis and interpretation of the results.
- Your samples will be analyzed using a 60 min peptide separation method (69€/sample (internal); 86.25€/sample (external)).