GUIDELINES AND INFORMATION FOR CUSTOMERS OF CFMP ZMBH
Title: Identification of protein interaction partners from pull-down experiments using in-gel approach

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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 pull-down/immunoprecipitation.**

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?
- Are you planning to use a detergent during cell lysis, washing or elution of the proteins?
- Did you already perform a test experiment? Did you see proteins on the SDS-PAGE gel when stained with Coomassie?

3. 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)
- For bulk identification of all protein partners, run your gel for **1 cm** (measure the distance from the bottom of the well to the running front). Then simply stop the electrophoresis, wash the gel intensively with dH_2O and stain with colloidal Coomassie.
- 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.

4. PROTEIN ELUTION FROM THE BEADS

If you work with the antibodies immobilized to the beads (cross-linked), then the most efficient way of releasing the proteins from the beads is to use 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.

Note: while working with **membrane proteins**, we recommend to warm sample up to 70 °C or test elution only in presence of SDS (without heating). Membrane proteins tend to aggregate while heated.

5. GENERAL INFORMATION

- We will provide you the results within 4 weeks from the sample submission
- For pull-down experiments, we recommend using at least 3 replicates per condition (e.g. 3 x bait and 3x control).
- Your samples will be analyzed using a 60 min peptide separation method (69€/sample (internal); 86.25€/sample (external)).