GUIDELINES AND INFORMATION FOR CUSTOMERS OF CFMP ZMBH

Title: Identification of protein interaction partners from Co-IP experiments

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1. Short description

Co-IP is employed to investigate protein-protein interactions within complex biological systems. The process involves the selective precipitation of a target protein along with its interacting partners using specific antibodies. Essentially, a protein of interest is immunoprecipitated from a cell lysate, along with its associated binding partners, forming a protein complex.

After the Co-IP step, isolated proteins might be separated on the SDS-PAGE and visualized using Coomassie staining. Once the proteins are visualized, the gel bands containing the proteins of interest are excised and subjected to enzymatic digestion, typically with trypsin. The resulting peptides are then analyzed using mass spectrometry. The in-gel approach allows to selectively omit heavy and light subunit of the antibody used for Co-IP (Protocol A). These subunits, which are usually present in excess, may hamper the LC-MS readout. If possible, we recommend using beads with covalently bound antibodies so that light and heavy subunits does not detach from the beads during protein elution. In such case, we recommend SP3 digest of the protein sample (Protocol B).

2. During initial meeting inform us about

- Do you already have iLab account?
- 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?
- Are antibodies covalently bound to the beads?

3. Sample preparation

Below, we provide two protocols suitable for efficient elution of the proteins from the beads. In both cases, elution buffer is based on the power of detergent (SDS) to disturb molecular interactions.

Protocol A is beneficial for processing of the samples, which might be contaminated by high excess of heavy and light antibody subunits. In this case, eluted proteins are separated on the SDS-PAGE.

Protocol B grants higher sensitivity and quantification accuracy.

Protocol A

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**.

Materials:

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	Fill up 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.

Recommendations for running the gel:

- 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 also run the gel in our facility**. We offer commercial gels (40€ / 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, reduced 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. De-stain the gel with water by incubating overnight.
- 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.

Protocol B

The most efficient way of releasing the proteins from the beads is to use SDS-based elution buffer. We recommend using **1x SDS-based elution buffer** similar to the one used for SDS-PAGE. Here is the recipe for **4x SDS-based elution buffer**.

Materials:

4x SDS-based elution buffer:

Component	Volume	Final concentration
Tris (1M, pH 6.8)	10 mL	200 mM
SDS	4 g	8%
B-mercaptoethanol	1 mL	2%
dH_2O	Fill up to 50 mL	

Elution procedure:

Mix beads with up to 40 µl of **SDS-based elution buffer** to reach final concentration of **1x SDS-based elution buffer**. An example: mix **40 µl of beads** with **20 µl of 4x SDS-based elution buffer** and **20 µl of 200 mM Tris pH 6.8**. 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-based elution buffer (without heating). Membrane proteins tend to aggregate while heated.

Hand-over the proteins for SP3 digest:

Please, contact **Ute Bach** from our facility to schedule an appointment for handing-over the proteins. Provide detailed composition and volume of the buffer used for protein elution.

4. 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.