GUIDELINES AND INFORMATION FOR CUSTOMERS OF CFMP ZMBH

Title: Protein identification from polyacrylamide gels (SDS-PAGE)

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1. Short description

Protein identification from SDS-PAGE involves a multi-step process to determine the identity of proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After proteins are separated based on size, they can be 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.

2. During initial meeting inform us about

- Do you already have an iLab account?
- Do you work with membrane protein?
- Protein length and specific features regarding protein sequence (amino acids sequence)
- What is the source of the protein?

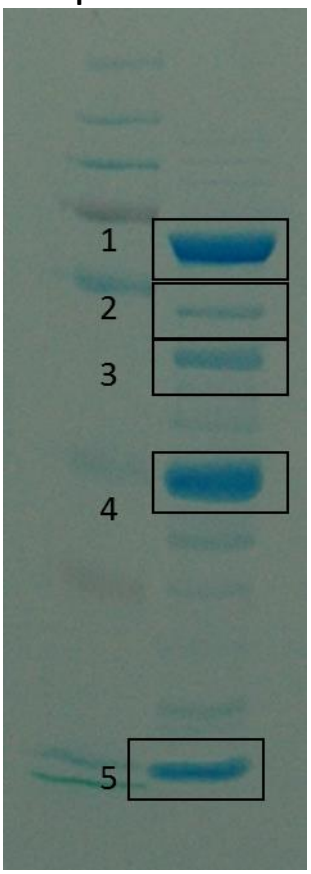
3. Sample preparation

- Use only detergents compatible with in-gel digest:
  SDS < 2%
  CHAPS < 4%
  NP-40 < 1%
- Use **commercial gels** (higher reproducibility and resolution, reduces contamination).
- 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.**
- For protein identification from the gel, run your gel for **full gel length**. 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 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.
- Follow the general guidelines for sample preparation to avoid excess of contamination.

Example:



- 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 make an appointment**.**

4. General information

- We will provide you the results within 4 weeks from the sample submission
- Your samples will be analyzed using a 30 min peptide separation method.