UNIVERSITY OF MICHIGAN MEDICAL SCHOOL
MICHIGAN MEDICINE
BRCF Proteomics & Peptide Synthesis Core

Sample Preparation Guidelines for Protein (Identification) Analysis

by Peptide Mass Fingerprinting, LC-MS or LC-MS/MS

Consult with the facility director BEFORE preparing your sample for submission.
We will provide important and project specific information on sample preparation and handling and determination of protein amounts. Sample submitted without prior consultation will not be processed. *Consultations are by appointment only.* Email: hremmer@umich.edu.

Commit as much protein as possible. The techniques used are **mole-based**, not mass based, i.e. with same staining intensity a 100kD protein contains **10x less** protein than a 10kD protein. The ***minimum protein amount necessary is 1pmol of protein in-gel*** (Commassie Blue stained!)

Follow the sample preparation procedure precisely to increase your chances for successful analysis.

For samples submitted in-gel: **only Coomassie Blue stain (e.g. Simply Blue colloidal Coomassie) is accepted** (silver stain may be used only after consultation with facility director and at the investigator's own risk). Stain as well as staining/destaining procedures must be mass spectrometry compatible and performed in a way to also minimize keratin contamination.

For samples submitted in –solution: SDS-PAGE electrophoresis (performed by the facility if desired) is mandatory.

Provide evidence of the protein amount present in your sample upon sample submission, e.g. by presenting the Coomassie Blue stained gel that contains a reference protein (e.g. BSA) of known concentration (1-5pmol). Reference proteins can be obtained at the Protein Structure Facility free of charge. Proteins submitted without valid evidence of amount/concentration will be subjected to SDS-PAGE (or Amino Acid Analysis if applicable) for an additional fee. If the protein amount cannot be determined due to the sample format (e.g. for excised stained gel bands submitted), the Protein Core cannot provide an estimation about the outcome of analysis and the laboratory PI's consent is needed by signature for sample submission.

In case the desired analysis result cannot be obtained due to the nature of the sample (e.g. insufficient protein amount submitted or sample is (keratin) contaminated) **charges for analysis steps performed apply in full.**

Director: Henriette Remmer, Ph.D.
Phone: 734.763.6285 Email: hremmer@umich.edu
Web: https://brcf.medicine.umich.edu/cores/proteomics-peptide-synthesis/
C570C MSRB II SPC 5613
1150 W. Medical Center Drive
Ann Arbor, MI 48109

SDS-PAGE and **2D gel electrophoresis** as **both acceptable** to prepare samples for protein mass spectrometry. It is important to maximize resolution as well as the protein –to-gel ratio (large amount of protein per gel volume).

Use a **gel thickness of 1.0 mm** and stain with Coomassie Blue (CBB) using a mass spectrometry compatible commercial solution or the following stain/destain:
Stain:0.5% acetic acid/20% methanol and CBB R-250 (stain for the minimum time necessary to detect the protein)
Destain: rinse the gel with 30% methanol in water

Excise protein bands sharply and include **a gel piece that represents the background** of the gel (the "gel blank") in a separate tube. Combine all lanes or spots for the same protein in **one** tube and wash the gel slices in the tube twice with 50% Acetonitrile/water. Submit the gel piece moist but without any excess of liquid. Your proteins are at this point not fixed in the gel and will diffuse into any excess liquid.

Minimize Keratin Contamination during sample preparation by wearing gloves, avoid touching your skin, rinsing all surfaces the gel (or sample) comes in contact with using deionized water and use a NEW (disposable) container for staining/destaining the gel, e.g. a large weigh boat.

The keratin background is always present. The less protein amount you provide, the more predominant this non-specific keratin background will be and the more success of analysis will be affected.

Necessary Protein Amounts:

Peptide Mass Fingerprinting: **minimum of 1pmol of protein** (in-gel,Coomassie Blue stained!)
LC-MS (e.g. intact protein*): **15 pmol/ul** (for MW up to 30kD) **in 20 ul minimum volume**
LC-MS/MS (protein ID): **minimum of 1pmol of protein** (in-gel,Coomassie Blue stained!)

*LC-MS of intact proteins can only be performed for proteins sufficiently small and hydrophilic to elute from C18-rp HPLC columns.

CORRECT SAMPLE PREPARATION, and ***SUFFICIENT PROTEIN AMOUNT*** are the

KEY FACTORS

for **SUCCESSFUL ANALYSIS**

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