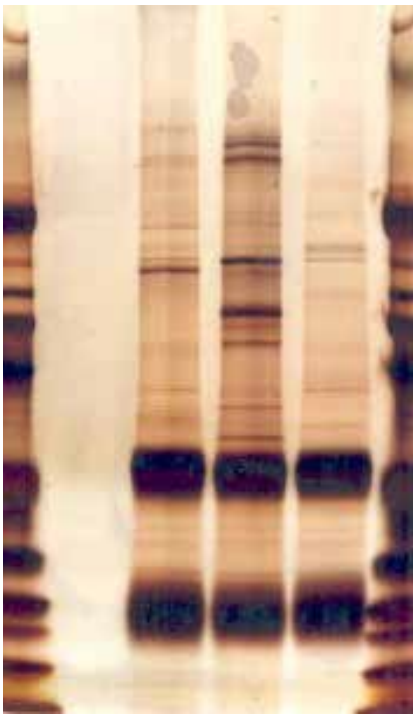
Fred Hutch Cancer Center

Proteomics & Metabolomics

Research Administration
Seattle, WA • 501(c)(3) Nonprofit

Fred Hutch's Shared Resources are catalysts for lifesaving discoveries. This uniquely centralized program of 15 specialized core facilities and scientific services drives advances by integrating dedicated experts and cutting-edge technologies across the entire research pipeline, from basic science to clinical trial.

SDS-PAGE Gel Handling Tips



1. Contamination of samples with keratin proteins is a constant battle in mass spectrometry labs. To help minimize this problem, wear gloves and a lab coat at all times when working with gels and their associated reagents.
2. Virtually any SDS-PAGE gel should be compatible with downstream protein identification. Acrylamide percentages do not matter. Pre-cast gels are suggested to help reduce keratin contamination. Pre-cast gels from Invitrogen and BioRad have been used routinely at the Hutch.
3. Use fresh staining reagents (contaminants build up in reused Coomassie stain). SimplyBlue SafeStain and GelCode Blue have been commonly used as commercial reagents for Coomassie staining. Silver staining and Coomassie staining are both compatible with mass spectrometry-based protein identification. Note: Silver staining kits should not contain glutaraldehyde as a fixing agent. Most commercial kits will state if they are MS-friendly.
4. After staining, wash gels thoroughly in ddH20 prior to band excision. Two washes at 15 minutes each should be sufficient. Longer washes are suggested for gels 1.5 mm and thicker.
5. Cut bands of interest from the gel with a clean, sharp razor blade. Cut the band directly on the edge of the stained region; no borders of clear acrylamide should be left around the band.
6. Place cut bands in Eppendorf tubes. (Other brands of tubes sometimes have contaminants that interfere with the mass spectrometry.) We suggest opening a new box of tubes and carefully selecting individual tubes with a gloved hand, again, to cut down on keratin contamination. We discourage the use of tubes from "community" containers.
7. The Eppendorf tubes can be stored at room temperature or at 4 degrees C until they are prepared. Storing the bands at -20 degrees C or -80 degrees C is discouraged, as water in the gel bands will expand and cause the gel pieces to disintegrate, making the in-gel digestion procedure more difficult and reducing recovery of digested protein.
8. Submit a blank region of the gel, approximately the size of the gel band of interest, with the sample(s). This will act as a blank control.
9. Label the tubes well (with initials, date and sample name).
10. Ship tubes via FedEx next-day air.

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Proteomics & Metabolomics
Phil Gafken, Ph.D.
Director
206.667.1051
proteomics@fredhutch.org

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Proteomics Solutions Handling Tips

There are many common biochemical reagents that are not compatible with mass spectrometry or HPLC and should not be present in the final sample. If the following reagents cannot be avoided during biochemical processing of the samples, contact the Proteomics & Metabolomics shared resource to develop a strategy for removing the problematic reagents.

- Detergents: NP-40, SDS, Tween and Triton-X are not compatible with LC/ESI, MALDI or HPLC.
- DMSO at high concentration is not compatible with LC/ESI, MALDI or HPLC.
- Phosphate buffers are not compatible with MALDI.
- Glycerol at high concentration is not compatible with MALDI.
- Salts (e.g., NaCl, KCl) at high concentrations are not compatible with MALDI.

If mass spectrometry-offending reagents are needed for protein isolation, one possible strategy is to remove the offending reagents by SDS-PAGE. Electrophorese samples 1-2 cm into a gel, stain the gel and cut out the band for downstream gel slice digestion processing (see figure 1 on next page). This gel approach is also ideal for removing excess FLAG peptide from FLAG-tagged affinity purifications.

Additionally, excess antibodies can interfere with mass spectrometry detection of lowabundance proteins. Either crosslinking the antibody to beads or fractionating the sample on a gel (see figure 2 on next page) can overcome this issue. It is important to note that bands cut from the gel should comfortably fit into an Eppendorf tube.

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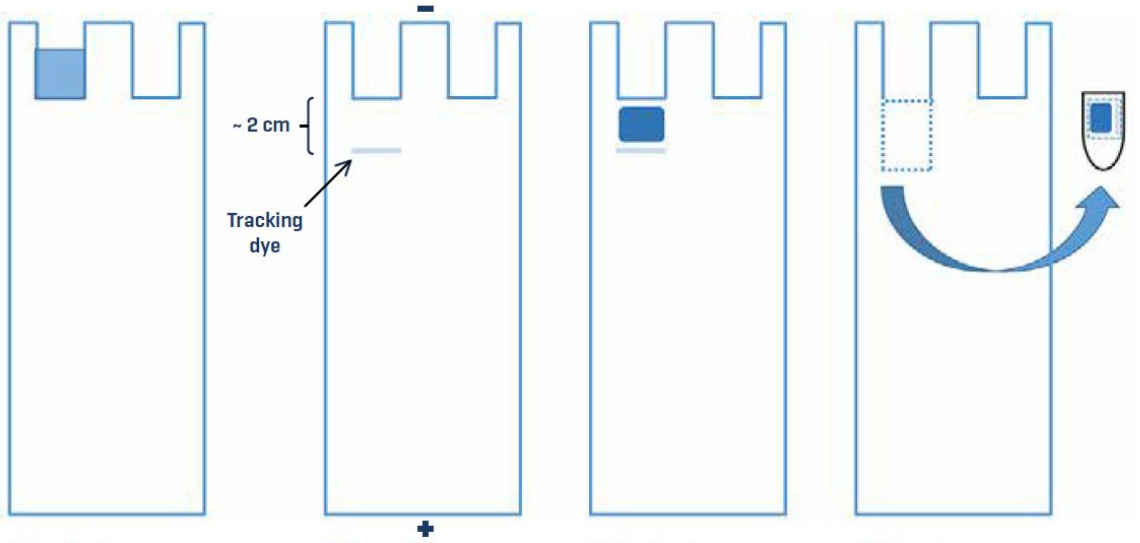
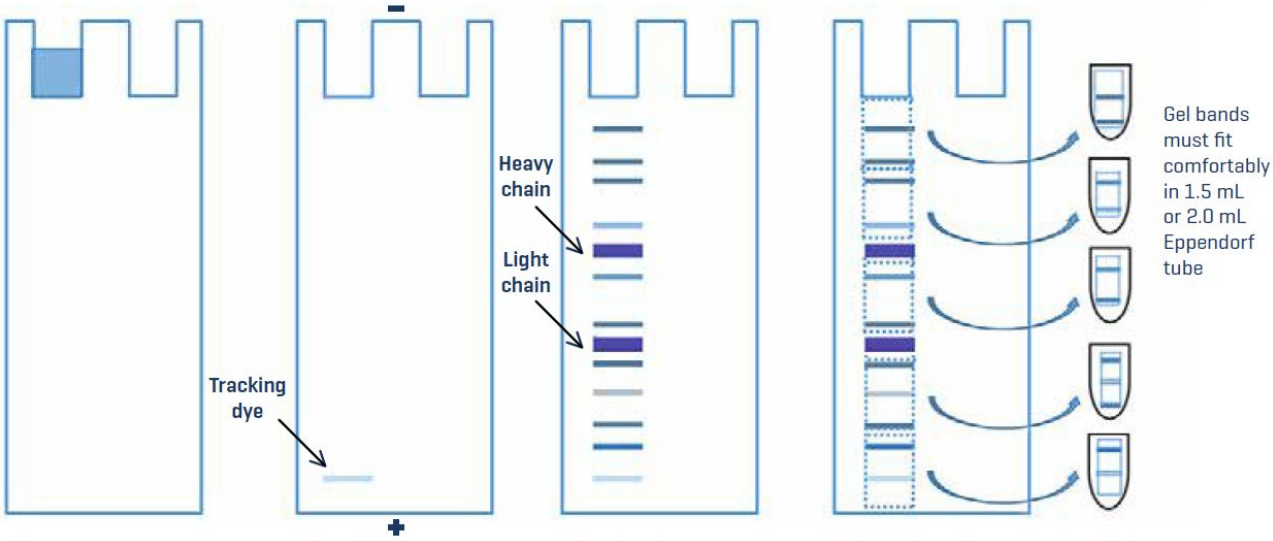
Figure 1: SDS-PAGE sample clean-up



1. Load gel
2. Run gel
3. Wash gel
   a. 50% MeOH/5% Acetic Acid
   b. Water (3x)
4. Stain gel (optional)
5. Cut region & place in Eppendorf tube

Figure 2: SDS-PAGE Fractionation - Antibody Present



1. Load gel
2. Run gel
3. Wash gel
   a. 50% MeOH/5% Acetic Acid
   b. Water (3x)
4. Stain gel (GelCode Blue, SimplyBlue, silver)
5. Cut regions & place in Eppendorf tube. Skip region/bands containing HC and LC
6. Submit to Proteomics & Metabolomics shared resource