Michael Hooker
UNC PROTEOMICS CORE

Avoiding Keratin Contamination

Keratin contamination is a common problem with mass spectrometry analysis. Sources of keratin contamination include contact with skin and hair, as well as dust, clothing, and latex gloves. If keratin is present in high concentrations, signal loss and a decrease in protein identifications may occur. Below are a few tips that will help minimize keratin contamination during sample preparation.

- Avoid storing gels in plastic wrap or similar material. Instead, use new and cleaned plastic or glass gel trays.
- Refrain from letting anything come into contact with the gel, such as dust or hair.
- Perform all sample preparation steps in a laminar flow hood, if possible.
- Wipe down surfaces with water and ethanol/methanol.
- ALWAYS USE NON-LATEX GLOVES and wear lab coats.
- Store consumables and reagents in covered containers, such as gels, liquid and powdered reagents, pipette tips, etc.
- No wool clothing in the lab.
- To avoid BSA or other contaminates from western blot assays: do not use the same plates or gel containers.
- For gel electrophoresis, wash all glass plates thoroughly with 70% ethanol prior to casting an SDS-PAGE gel. After completing gel electrophoresis step, disassemble the glass plates in a laminar flow hood. Destain the gel in a clean container that has been rinsed thoroughly with 70% ethanol or methanol/acetonitrile.

Michael Hooker
UNC PROTEOMICS CORE

Preferences for gel-based sample submissions

- We recommend using precast gels (NuPAGE gels, Invitrogen) or (Bio-Rad Mini-PROTEAN® TGX™ Precast Protein Gels, Bio-Rad Stain Free Gels), pre-mixed buffers (MES or MOPS SDS Running Buffer, Invitrogen/Bio-Rad), and pre-mixed loading buffer (NuPAGE LDS Sample Buffer or Laemmli sample buffer, Invitrogen/Bio-rad).
- For better protein separation use gradient gels.
- Stain gels with any of the following: Biorad Bio-Safe™ Coomassie Stain, colloidal coomassie G-250, or pre-mixed colloidal blue staining solution (Invitrogen). If you need a more sensitive stain, we recommend Sypro ruby stain. Take a UV gel image before submitting the gel.
- Store gels in DI water or 3% acetic acid, at 4°C.
- Gels submitted for binding partner ID analyses should be run maximum 1 cm sample lane length and **lightly** stained with coomassie.

Preferences for in-solution sample submissions

- If detergents are necessary during sample preparation, please consider using RapiGest (Waters Corp) or another MS-compatible surfactant.
- All other detergents are not MS-compatible and must be removed prior to sample submission.
- Please determine the protein concentration using a BCA or Bradford assay prior to sample submission and include this information on the sample submission form.