THE WISTAR INSTITUTE

PROTEOMICS AND METABOLOMICS FACILITY
Hsin-Yao Tang, Ph.D.
Managing Director
P (215) 898-3830 E tangh@wistar.org

Gel Shipping Instructions

Please note that we require that the entire gel be sent to us. We strongly recommend the gel be packaged and shipped per the instructions noted below. Gel(s) that are not securely packaged prior to shipment usually are severely damaged or destroyed during shipment due to abuse of the package by the shipper. We have found the following packaging technique to be successful (gel arrives intact):

1. Take a picture of the gel, mark on the copy the bands of interest, and include this with the submission sheet in a separate plastic bag in the shipping box—packed outside the ice (see further instructions regarding the use of ice).
2. Place the gel in a watertight Ziploc® plastic bag after draining all excess water from the gel. The gel should remain moist with only a few drops of water in the bag. The gel should not be floating in the bag.
3. Cut two pieces of cardboard slightly larger than the size of the bag containing the gel. Place the gel and bag between the cardboard covers and tape all sides.
4. Securely wrap the cardboard/gel+bag/cardboard sandwich with bubble wrap.
5. Place the entire bubble-wrapped sample on top of or near the top of loose, wet ice in a Styrofoam™ shipping container and send to us by the overnight courier of your choice. DO NOT use icepacks or pack bubble-wrapped sample between layers of wet ice, as this may freeze the gel and result in breakage.
6. Send an email message to Dr. Hsin-Yao Tang (tangh@wistar.org) the day the package is shipped. We will alert you if we do not receive the package by 4:00 p.m. the following day. Do not ship packages on a Friday afternoon for Saturday or other weekend/holiday delivery.

SHIP TO:
Wistar Proteomics and Metabolomics Facility
Attn: Hsin-Yao Tang, Ph.D.
3601 Spruce St., Room 252
Philadelphia, Pa. 19104-4268

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Sequence-quality SDS-gel guidelines for MS Analysis

Preparing Samples and Gels

1. Maximize protein concentration in the gel. High quality pre-made commercially available mini gels (1.0 mm thick) such as the ThermoFisher NuPAGE gels are strongly recommended. In general, follow the manufacturer's guidelines.
2. Select a gel type and separation buffer that will give a good distribution of proteins throughout the gel for complex samples or sharp, tight bands for the proteins of interest with an Rf between 0.3 and 0.7 if a single or several bands will be excised for analysis.
3. Avoid airborne contamination throughout sample preparation and electrophoresis.
4. Solubilize samples using 2X or 5X solubilizing buffer containing sucrose or glycerol. **Do not use buffers containing urea!**
6. Do not heat samples excessively. If higher temperatures are needed to properly solubilize sample, minimize as much as possible, e.g. 1-2 minutes at 80-90^{o}C.

Separation and Staining of Gels for In-Gel Digestions

1. Avoid excessive heating (>25^{o}C) during electrophoresis.
2. **Do not** run bromophenol blue dye front off the bottom of the gel. This will not improve the separation.
3. After electrophoresis immediately stain with either a) ThermoFisher Colloidal Blue (catalog # LC6025 - follow manufacturer's guidelines for staining and destaining); or b) ThermoFisher SilverQuest™ (catalog # LC6070 - follow manufacturer's guidelines for staining and washing. Do not destain).
4. Seal destained gel (do not over-destain) in a Ziploc® bag and store at 4^{o}C (add only a few drops of water to the bag, gel should not be floating).
5. The entire gel must be sent to us; follow "Gel Shipping Instructions."
6. Include photocopy or picture of gel with desired band(s) for digestion clearly marked.

General Gel Comments

- Urea decomposes readily to form cyanate, which will modify your protein at higher temperatures and pH above 7. It is advisable to eliminate urea from your protocol when doing gels for sample preparation.
- Heating during sample prep or electrophoresis can contribute to chemical modifications of your protein and therefore should be minimized.
- **DO NOT run the tracking dye off the gel!** Instead, change the gel %, if needed, for optimal separation results.