PROTEIN IDENTIFICATION

The Redox Molecular Signaling Core utilize Waters SYNAPT HDMS to identify proteins from SDS-PAGE gel bands. To initiate Protein Identification, contact the Redox Molecular Signaling core facility at **RedoxMolSignalCore@lsuhsc.edu** and schedule a meeting with Core Leaders to discuss the project timeline and deliverables.

To be provided by investigator:

- A completed Work Order Form brought to the meeting with Core Leaders.
- The Gel band samples which are prepared according the following SOP.

To be generated by the core:

- Reduction/alkylation/Trypsin digestion and peptides extraction
- Protein identification LC-MS report

Timeline: Timeline may depend on sample number and core schedule. Typical timelines range around 2 weeks.

Equipment and materials:

- Coomassie blue R-250 (Sigma-Aldrich, cat. No.27816)
- Acetic acid (Sigma-Aldrich, cat. no. 695092)
- Low Protein Binding Collection Tubes (ThermoFisher, Cat. No. 90410)

Procedure (Note: wear gloves at all times to reduce sample contamination):

- Staining the SDS-PAGE
    - After electrophoresis, fix gel in the fixing solution (50% methanol and 10% glacial acetic acid) for 1 hr with gentle agitation
    - Expose the gel in staining solution (0.25% Coomassie Brilliant Blue R-250, 50% methanol and 10% glacial acetic acid) for 20min~4h with gentle agitation. Note: Not all Coomassie stains are compatible with LC-MS analysis, so be sure to verify the compatibility prior to staining. Always try to only stain your gel for the minimum time to visualize your interest band because extended staining will increase the background in subsequent MS analysis, and not increase the amount of protein.
    - Destain gel in destaining solution (5% methanol and 7.5% glacial acetic acid), and change the solution several times until background of the gel is fully destained.
- Scanning of gel bands
    - Put the gel into the cover of the 14-cm dish and scan it.
- Cutting of gel bands

- Put the gels on a clean glass plate. Cut away the unnecessary parts (top, bottom, MW marker lanes) with a new clean razor blade or scalpel.
- Precisely cut out ONLY interest band (any excess gel will lead to background noise). Chop the excised bands into cubes (ca.2×2 mm)
- Transfer these cubes into a new low protein binding Collection Tubes (1.5 ml). Spin them down on a bench-top microcentrifuge. Label the tubes. For short term storage, place the tubes at 4°C for a few weeks. For long-term storage, place the tubes at -20°C for a few months.