Gel Coomassie Staining Protocol  
University of Utah Proteomics Core Facility

Reagents

*All reagents must be made up with nanopure water*

Coomassie Blue Stain:
- Dissolve 400 mg of Coomassie blue R350 in 200 mL of 40% (v/v) HPLC grade methanol in water with stirring as needed.
- Filter the solution to remove any insoluble material.
- Add 200 mL of 20% (v/v) acetic acid in water.
- The final concentration is 0.1% (w/v) Coomassie blue R350, 20% (v/v) methanol, and 10% (v/v) acetic acid.

Coomassie De-stain: 10% methanol, 7% acetic acid in water

Wash Solution: 50% methanol and 5% acetic acid in water

Protocol

Resolve proteins by SDS-PAGE (after running the protein on the gel everything must stay clean to avoid contamination)  
- Save the original plastic Bio-Rad™ tray or have a designated proteomics tray to coomassie stain and destain your gel.

- Rinse the tray with water and clean with 70% ethanol and rinse with water before placing your gel into the tray

- Rinse the gel twice, rocking gently for 10 minutes with water to remove excess SDS-running buffer
  - Cover the tray with saran wrap to prevent contamination

- Stain the gel with Coomassie brilliant blue for 1 hour rocking slowly at room temperature  
  - Coomassie stain should be new or used only for proteomics gel stains

  - Cover the tray with saran wrap to prevent contamination

- De-stain the gel overnight with 10% methanol and 7% acetic acid solution. Change the de-stain solution after 1 hour and 2 hours before leaving overnight  
  - Cover the tray with saran wrap to prevent contamination

- On a clean surface (wipe down a small square of glass with 70% ethanol) cut the protein bands from the gel and place in wash solution overnight.

  - Continue to change wash solution 3x/day until protein bands are clear.

Gel pieces can be stored at room temperature in 300 µL of wash solution until they are submitted to the Proteomics Core Facility.