**Destaining Sypro Ruby/Deep Purple/Coomassie G250 Gel Plugs and Bands**

1. Run gel. If possible, use a precast gel. If precast gels are not an option, filter acrylamide solutions prior to pouring the gel and wash and dry glass plates in a dishwasher. Avoid using Kimwipes. If wipes are needed, use low linting wipers such as Kimtech Critical Task Wipers, which can be purchased from VWR (cat. no. 21908-205). Run one lane with 0.5-1 ug BSA to be used as a positive control. If doing an in-gel trypsin digestion for the first time, I can provide a BSA positive control.

2. Stain gel. To get the best mass spectrometry results, avoid using silver staining. If choosing Coomassie Brilliant Blue as your staining reagent, use Colloidal Coomassie G250 rather than R250. A number of vendors now sell colloidal coomassie staining solutions: BioRad (Bio-Safe), Pierce (GelCode Blue), Invitrogen (SimplyBlue SafeStain).

3. Pick spots/cut bands. Working in a dust-free environment and wearing gloves and a lab coat, pick spots using a clean Pasteur pipet/cut-off pipet tip, or cut out gel band using a clean razor blade. Rinse cutting tool with dI $H_2O$ before touching gel, and avoid using Kimwipes. If the gel is stained with Sypro Ruby or Deep Purple, spots can be picked using a standard UV lamp. Place gel plugs in clear polypropylene tubes/plates. If working with gel bands, cut the band into 2 mm slices and combine into one tube.

4. Destain gel plugs/bands.
    a. Incubate 2.0 mm gel plugs with 100 µl 50 mM ammonium bicarbonate in 50 % methanol for 20 minutes at room temperature. If working with gel bands, increase volume relative to gel amount (I typically use 1 mL, and place on a rocking platform.). If using Coomassie, increase destaining time to remove as much stain as possible.

        79 mg ammonium bicarbonate
        10 mL methanol
        10 mL $H_2O$

    b. Remove methanol solution. Repeat. If using Coomassie, repeat until methanol solution is no longer blue.

5. Dehydrate/dry gel plugs/bands.
    a. Incubate 2.0 mm gel plugs with 100 µl 75 % acetonitrile for 20 minutes at room temperature. Again, if working with gel bands, increase volume relative to gel amount.

        15 mL acetonitrile
        5 mL $H_2O$

    b. Remove acetonitrile solution. Dry gel plugs at 40 $^oC$ for 15-20 minutes. If working with gel bands, increase drying time to ensure that gel is completely dehydrated and “crisp”.