**Preparation of Gel Bands for In-Gel Digestion**

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**Before you start your project!!!**

* All users must contact Dr. Kakhniashvili and discuss specific project details before submitting samples to the PMC
* Read the Sample Preparation Basics SOP for the PMC

**SDS PAGE Gels**

Use either pre-cast or home-made polyacrylamide gels, high-grade chemical reagents, and fresh buffers. The size of a band to be excised from a gel should not exceed these dimensions: 1mm X 1mm X 5mm. Therefore, use gels with 1 mm spacers (gel thickness) and 4-6 mm wide wells. Before use, leave any home made gels overnight on the bench at **room temperature** to complete polymerization step and to prevent protein crosslinking by residual radicals in the gel; during this step you must prevent the gels/wells from drying.

**Coomassie Staining/Distaining**

1. **Do not** use ‘instant’ or ‘ready-to-use’ staining kits (“InstantBlue” or “EZBlue” reagents)
2. Use only fresh **unused** colloidal Coomassie stain (GelCode Blue, Thermo) or a proteomic compatible stain, such as Sypro Ruby (Thermo or Bio-Rad)
3. Try to stain gels for the minimum time needed until the band(s) of interest are visible
4. De-stain bands sufficiently to clearly see the band of interest edges
5. **Do not use** a microwave to speed up staining or distaining
6. Save a high-quality image of the gel prior to band excision (optional)
7. NEVER place gels on plastic for cutting , only cut gels on a clean glass plate

**Excising the Band of Interest from a Gel**

1. Cut gels on a clean glass plate **ONLY**
2. Wear gloves at all times
3. Use a **new** scalpel blade and clean forceps during cutting
4. **Precisely** cut out **only** the clearly stained band, which must not have diffused edges
5. **If band is wide, cut out its darkest, middle strip no wider than 1 mm**
6. Cut the gel band into 2-3 pieces – **BUT, do not mince** into small pieces
7. Gel band pieces **should not be allowed to dry**. Put the excised pieces of a band into a clean 1.5 ml Eppendorf tube immediately and cap the tube tightly to keep the gel pieces moist.
8. Gel band pieces are now ready for in-gel digestion and can be stored for 2-3 days at 4°C or six months at -20°C for further processing
9. Wear gloves at all times