Excision of Protein Bands/Spots from Polyacrylamide Gels

1. Purpose

This protocol describes the excision of stained protein spots/bands from a polyacrylamide gel. The excised gel can be submitted for protein identification by mass spectrometry.

2. Reagents and Materials

2.1. 1.5 ml (or 0.5 ml) microcentrifuge tubes, siliconized (or low protein-binding)
2.2. Acetic acid
2.3. Scalpel with fine-sized blades (*e.g.* #11 surgical blade)
2.4. Clean work area (e.g. positive pressure clean hood or enclosed laminar flow hood)
2.5. 90% ethanol
2.6. 70% ethanol
2.7. Water (Milli-Q or Optima grade, or similar)
2.8. Suitable cutting surface (*e.g.* light box, glass plate), very clean
2.9. Clean, non-latex gloves

3. Reagent Preparation

3.1. 1% Acetic Acid
 3.1.1. Add 10 µl of glacial acetic acid to 990 µl water (assume 1 ml per 10 gel bands).
 3.1.2. Vortex to mix.
3.2. Clean tubes
 3.2.1. Clean the microcentrifuge tubes with 90% ethanol (or methanol) and dry completely.

4. Procedure

4.1. Thoroughly clean the cutting surface with 70% ethanol (or methanol) and place it into the clean work area.
4.2. With a clean blade, excise the protein band or region of interest.
 4.2.1. If cutting a single band (rather than a multi-band region), do not include any non-stained polyacrylamide. If the band is large, excise only the darkest central portion.
 4.2.2. Push the blade straight down to avoid creating rips or tears.
4.3. Place the cut gel into a pre-cleaned microcentrifuge tube and add sufficient 1% acetic acid to cover the gel (if commencing an in-gel digest immediately, water can be used instead).
4.4. If cutting a large number of spots, add water to the gel as needed to prevent drying. A squirt bottle filled with milli-Q water is handy for this purpose. Dry gel will curl and may rip.
4.5. Store at 4°C for short-term or freeze for long-term.

5. Notes

5.1. Extreme care must be taken to avoid contaminating the gel with keratin (the major protein of skin and hair) or other environmental proteins. A lab coat with elastic or fitted cuffs should be worn. Wool clothing should not be worn. Clean non-latex gloves must be worn at all times. Prior to cutting, the gel should be kept covered at all times.

5.2. Use only the highest-purity reagents for proteomics experiments. Commercially precast gels are often cleaner than lab-made gels.

5.3. If the equipment or glassware used to run the gel is also used for Western blots, take great care to avoid cross-contamination from Western blotting reagents, especially "blocking" proteins such as skim milk powder or serum albumin.