

# **Nondestructive Silver Staining of Polyacrylamide Gels for Mass Spectrometry**

**Working Detection Range:** 10-50ng of protein

*Procedure:*

All steps are done on a rotary shaker with gentle mixing

1. Fix the gel for two hours in 40% methanol/ 10% acetic acid immediately following electrophoresis. This step can be done overnight if it is more convenient.
2. Rinse the gel with distilled water until all fixative has been removed. Typically this requires three water changes of one hour duration each. Gels can be left at this step overnight if required. More extensive washing will result in a lower final gel background.
3. Sensitize the gel in 0.02% sodium thiosulphate for two minutes.
4. Rinse the gel twice in distilled water for one minute each to remove excess thiosulphate solution.
5. Stain the gel with 0.1% silver nitrate for 30 minutes.
6. Rinse the gel twice in distilled water for one minute each to remove excess silver reagent.
7. Develop the gel in a solution of 0.04% formaldehyde/ 2% sodium carbonate. Protein features should appear in approximately 5 minutes.
8. When a sufficient amount of staining has occurred, stop the developing reagent by placing the gel in a solution of 5% acetic acid for 30 minutes.
9. Gels can be stored in 1% acetic acid at 4C for months.

Reference: modified from A. Schevchenko et al. Anal. Chem., 68, 850-858, 1996