

Silver Staining Protocol Compatible with Mass Spectrometry

Modified from "Mass Spectrometric Sequencing of Proteins from Silver-Stained Polyacrylamide Gels" A. Shevchenko, M. Wilm, O. Vorm, M. Mann *Analytical Chemistry* **1996**, 68 (850 – 858).

I. Convenient Recipes

- a. **Sensitization Solution** (0.02% sodium thiosulfate) – 0.1 g sodium thiosulfate in 500 mL MilliQ water
- b. **Staining Solution** (0.1% silver nitrate) - 0.5 g silver nitrate in 500 mL MilliQ water (use glass container only!)
- c. **Developing Solution** (0.04% formalin in 2% sodium carbonate) - 125 μ L formalin (37% formaldehyde solution) and 15 g sodium carbonate in 500 mL MilliQ water

II. Staining Procedure

- a. Immediately following electrophoresis, remove gel from plates and notch the upper right corner. Place gel in the **fixing solution** (50% methanol: 12% glacial acetic acid) and gently shake overnight.
- b. Discard the fixing solution. Wash the gel with 50% methanol for 20 minutes.
- c. Discard the wash solution. Wash the gel with MilliQ water for 20 minutes.
- d. Repeat wash in step IIc.
- e. Discard wash solution. Place gel in **sensitization solution** (0.02% sodium thiosulfate) for 1 minute.
- f. Discard sensitization solution. Wash the gel with MilliQ water for 1 minute.
- g. Discard wash solution. Wash the gel a second time in MilliQ water for 1 minute.
- h. Discard the wash solution. Place the gel in the **staining solution** (0.1% silver nitrate, chilled) for 20 minutes.
- i. Discard the staining solution. Wash the gel with MilliQ water for 1 minute.
- j. Discard the wash solution. Wash the gel a second time with MilliQ water for 1 minute.

- k. Discard the wash solution. Place the gel in the **developing solution** (0.04% formalin in 2% sodium carbonate). Time is variable depending on desired staining intensity.
- l. Discard developing solution. Place gel in **stopping solution** (5% glacial acetic acid) for 10 minutes.
- m. Discard stopping solution. Store gel in 1% glacial acetic acid at 4°C.

Notes:

- Wear clean non-latex gloves at all times to avoid keratin contamination.
- Wash Pyrex dish(es) with detergent and wash with copious volumes of deionized water prior to starting silver staining protocol.