

Silver Staining of Protein gels

Materials:

- Gel to stain
- One clean plastic/glass tray that can comfortably hold 100ml solution on a rotatory shaker
- Two 250ml glass conical flasks
- One 100ml measuring cylinder

Chemicals:

- Silver Nitrate
- Sodium carbonate
- Sodium thiosulfate
- Formaldehyde
- Methanol or Ethanol
- Acetic Acid
- Sufficient H₂O

Solutions to make:

1. Fixer 1: 50% methanol (or ethanol) v/v in H₂O with 0.05% formaldehyde
 - 50ml Methanol (or ethanol), 50ml Water, 135 μ l 37% formaldehyde
2. Fixer 2: 25% methanol (or ethanol) v/v in H₂O with 0.05% formaldehyde
 - 25ml Methanol (or ethanol), 75ml water, 135 μ l 37% formaldehyde
3. Stain: 0.2% Silver Nitrate solution (w/v; 100ml in H₂O) with 0.076% formaldehyde
 - 0.2g AgNO₃ dissolved in 100ml water, add 205 μ l formaldehyde
4. Sensitizer soln (0.02% Sod. Thiosulfate w/v; 100ml in H₂O)
 - Dilute 1:100 from a 2% stock solution (0.2g in 10ml water): 1ml stock + 99ml water
5. Developing soln (6% sodium carbonate in 100ml H₂O ; but see procedure below)
 - Weigh out 6g sod. Carbonate, add 2ml Sensitizer solution, add water to 100ml, swirl to dissolve.
6. Stop soln (10% methanol [or ethanol]-5% Acetic acid v/v in H₂O)
 - 10 ml methanol (or ethanol), 5ml acetic acid, 85ml water

Procedure:

- **Gel Fixation:** After electrophoresis, place gel in a tray containing 100ml of Fixer 1, shake for 30min (to O/N; shaking optional)
- Decant; add Fixer 2, shake for 20 min
- Wash with H₂O 3x5min
- Meanwhile, prepare Sensitizer solution and the Stain in two 250ml conical flasks (label accordingly)
- **Sensitization:** Decant H₂O, pour ~98ml sensitizer to the gel (leaving behind ~2ml soln in the flask). Shake gel for 1min. Decant sensitizer and wash gel 3x1min with H₂O
- **Staining:** Add stain to the gel. Cover tray with plastic wrap (optional), shake for 25min
- Meanwhile, prepare Developing soln by adding 6g Sod. Carbonate to ~2ml sensitizer solution (saved from Sensitization step above) and dissolving to a final 100ml with H₂O. Swirl to help speed up dissolution.
- Keep the Stop solution ready.
- Drain Stain into a separate container (*do not drain into the sink*) meant for liquid chemical disposal. Wash gel with H₂O 3x1min with excess water (~100ml)
- **Stain Development:** Add 150 μ l Formaldehyde to the Developer solution., mix well by gentle swirling. Add to the gel. Keep shaking the gel-tray gently and observe stain development. Depending on the amount of proteins, stain development can happen from a few seconds to several minutes. Once desired intensity obtained, decant the developer solution and add the Stop solution promptly, or the bands can get too coloured to be analyzed. It may be desirable to decant Stop solution after a while and pour some fresh. Store gel preferably in dark and at 4C, or store dry.

This protocol is compatible with MALDI-MS/MS analysis of protein bands.