Silver Staining

Silver Staining optimized for sensitivity and mass spectrometry compatibility.

This protocol is adapted from Mortz et. al. (*Proteomics, 2001, 1 1359-1363*).

If the gel is to be used for in gel trypsin digestion and protein identification, see the general gel handling procedures described in the In Gel Digestion procedures.

Materials

- Milli-Q water
- Acetic Acid
- Ethanol
- $Na_2S_2O_4$ – sodium thiosulphate
- $AgNO_3$ – silver nitrate
- Formalin or formaldehyde
- Na_2CO_3 – sodium carbonate

Solutions of sodium thiosulphate and formaldehyde should be made fresh. Solutions of acetic acid and ethanol can be made in advance and stored as stock solutions. Silver nitrate can be maintained at a 10x stock solution at 4C in the dark, then diluted prior to use.

For procedures, exact volumes are not given as these will vary of the size of gel and the glass ware used.

Procedure

1. Fix the gel for 1 hour in a solution of 40% EtOH and 10 % acetic acid.
2. Wash the gel 2 x 15 minutes in 30% EtOH.
3. Wash the gel for 15 minutes in ddH_2O.
4. Sensitize the gel for 1 minute in 0.02% $Na_2S_2O_3$.
5. Wash the gel 3 x 20 seconds in ddH_2O.
6. Wash the gel in for 20 minutes in 0.1% $AgNO_3$ at 4°C.
7. Wash the gel 3 x 20 seconds in ddH_2O.
8. Transfer gel to a clean tray
9. Wash the gel for 1 minute in ddH_2O.
10. Develop the gel in 3% Na_2CO_3 and 0.05% formaldehyde or formalin. Follow the colour change of this developer solution, if it turns yellow add fresh solution. Terminate the staining when it is deemed sufficient.
11. Rinse in ddH_2O.
12. Stop staining by washing in 5% acetic acid. Incubate for at least 30 minutes.
13. The gel can be rinsed with ddH_2O and stored in 1% acetic acid at 4°C until further use (drying or for protein ID).

Note: Be sure to dispose of all methanol containing solutions appropriately. Methanol should never be disposed of down the drain.