Arizona Proteomics Consortium Protocol
08/26/2014

Silver Staining Method Compatible with Mass Spectrometry

(Adapted from Blum, et al., Electrophoresis 8, 93, 1987 and Shevchenko, et al., Anal Chem 68, 850, 1996)

Equipment:

Shaker table
Dedicated trays for silver staining

Solutions:

Use the highest grade of chemicals/reagents available for all steps.
Prepare Sensitizer, Silver and Developer reagents **fresh** every time.

Fix 1:	50% methanol/10% acetic acid
Fix 2:	5% methanol/1% acetic acid
Fix 3:	50% methanol
Stop:	6% acetic acid or 40 mM Na_2-EDTA

SENSITIZER: 0.02% sodium thiosulfate
20 mg $Na_2S_2O_3 \bullet 5H_2O$ + 100 ml ddH_2O

Reserve a small volume of SENSITIZER (2.0 mL) for DEVELOPER solution.

SILVER: 0.2% silver nitrate
200 mg $AgNO_3$ + 100 ml ddH_2O Keep at 4ºC until used

DEVELOPER: sodium carbonate/sodium thiosulfate/formaldehyde
6.0 g $NaCO_3$ + 2ml SENSITIZER (0.02% $Na_2S_2O_3 \bullet 5H_2O$) + 50 µl (37% stock) formaldehyde + 100 ml ddH_2O (it may take a moment for the Na_2CO_3 to dissolve). Important: for the clearest background add the formaldehyde just before starting the development step.

Method

Begin protocol immediately following electrophoresis

Following incubation, SILVER must be discarded in hazardous waste container designated for metals. DO NOT discard in sink.

1. Incubate gel in Fix 1 for 30 min with shaking.
2. Incubate gel in Fix 2 for 15 min with shaking.
3. Rinse gel in Fix 3 for 1 min (optional, use for larger gels, 18 –24cm).
4. Wash gel in ddH_2O with shaking, at least 3 x 10 min.
5. Sensitize exactly 90 sec in SENSITIZER solution, with shaking.

6. Rinse gel in ddH_2O 3 x 30 seconds.
7. Incubate gel in chilled SILVER solution for a minimum of 30 min, with shaking. Gel can be left in SILVER solution for extended periods at 4ºC (e.g., overnight.).
8. Remove SILVER solution from gel and discard in an appropriate hazardous waste container.
9. Rinse gel 3 x 1 min **Absolute Maximum**
10. Add 50 ul of formaldehyde to the DEVELOPER solution.
11. Incubate in DEVELOPER solution, with shaking. Protein stain will become visible within 1-5 minutes, depending upon the total protein concentration; develop gel to desired intensity.
12. Stop with 100 mL 6% acetic acid or 40 mM EDTA, shake for 5 min.
13. Wash gel in ddH_2O for a few minutes.
14. Cut out and destain bands ASAP after staining the gel, follow the silver destaining protocol below.

Destaining Silver Stained Gel Bands Prior to Protease Digestion

Stock solutions

Use the highest grade of chemicals and water available.

60 mM potassium ferricyanide
198 mg potassium ferricyanide + 10 ml H_2O
This solution can be stored in an amber colored glass or plastic bottle for several months. Mix 1:1 with the 200 mM sodium thiosulfate solution only when ready to destain bands/spots.

200 mM sodium thiosulfate
316 mg sodium thiosulfate + 10 ml H_2O
This solution can be stored in a clear colored glass or plastic bottle for several months. Mix 1:1 with the 60 mM potassium ferricyanide solution only when ready to destain bands/spots.

Method

1. Destain the band using 30 mM potassium ferricynide/100 mM sodium thiosulfate solution until the brown color disappears. Use sufficient solution to cover the band. The solution may need to be changed if it takes longer than 5 minutes to destain. DO NOT let the band sit in this solution too long.
2. Remove the destain solution and wash the band for 5-10 minutes at least 5 times with H_2O, the solution should be clear.
3. If the sample will not be digested right away, store in water or freeze the gel band.