

Silver Staining Gel Protocol Compatible with Mass Spectrometry Analysis

Reagents:

Ethanol	Acetic Acid
Sodium Thiosulphate	Sodium Acetate
Silver Nitrate	Sodium Carbonate
Formaldehyde	
Potassium Ferricyanide	Sodium Thiosulfate

Silver Staining Solutions: *(make fresh each time)*

Fixing Solution: 40% ethanol/10% acetic acid

Sensitizing Solution: 30% ethanol/.2% sodium thiosulphate/6.8% sodium acetate

Silver Nitrate Solution: 0.25% Silver Nitrate in Water

Developing Solution: 2.5% Sodium Carbonate/.015% Formaldehyde

Add Formaldehyde immediately before use

Stop Solution: 5% Acetic Acid

Destain Solutions:

30 mM Potassium Ferricyanide: 9.876 mg/ml *(store for 3 months @ 4°C)*

100 mM Sodium Thiosulfate: *(store for 3 months @ 4°C)*

Farmer's Reducer: *(make fresh for each use)* combine above in a 1:1 mixture, to make 15 mM K-Ferricyanide and 50 mM sodium thiosulfate

Make ALL solutions fresh on day of staining. The silver solution needs to be kept wrapped in foil or in the dark until it is used. It is light sensitive and only good on the day it is made.

Gel Staining Protocol:

1. Place the gel in a container with **Fixing Solution** and shake for at least 1 hour. Ensure that the **Fixing Solution** covers the gel
2. Drain the fixing solution from the container
3. Add **Sensitizing Solution** to the gel and shake for 30 minutes
4. Drain sensitizing solution from the container
5. Wash 3x with dd water (5 minutes each time)
6. Add **Silver Nitrate Solution** and shake for 20 minutes
7. Put **Silver Nitrate Solution** into a hazardous waste beaker
8. Wash 2x with dd water (1 minute each time)
9. Add **Developing Solution** to the gel and shake until yellow or brown "smokey" precipitate appears. Pour off developing solution and add fresh developer, continuing as needed until desired intensity of spots is achieved
10. Drain **Developing Solution** and add **Stop Solution**, shaking for 10 minutes
11. Wash 3x with dd water (5 minutes each time)
12. Acquire the image and store the gel at 4°C for several months

Processing the Gel for Mass spectrometry Analysis

1. Bands will be cut out by the Proteomics Staff, diced into 1 mm squares and placed in LoBind tubes
2. Oxidize the gel pieces, using 100 µl of **Farmer's reducer** for 10 min @ RT
3. Remove Farmer's Reducer and wash gel slice three times (for 15 minutes each time) with 400 µl dH₂O over a total of 45 min @ RT
4. Remove water and subjected the gel fragments to the standard in-gel digestion protocol.