Modified Silver Staining of polyacrylamide gels

The proteins are separated by 1D or 2D SDS-PAGE, silver stained and analyzed by mass spectrometry.

All steps are performed on a shaking table at room temperature except step 5.

1. Fix gel with destaining solution

• Methanol: acetic acid: water (27.5%: 5%: 67.5%)

• Time: 20-30 min

2. Rinse gel with water to remove acid

• Rinse the gel slab with water (Mili-Q grade3 changes, two minutes per change) and then leave it in water for one hour.

3. Sensitize gel with 0.02% (w/v) sodium thiosulfate

• 0.1 g sodium thiosulfate in 0.5 L water

• Time: 1-2 min

4. Rinse gel with two changes of water (10 seconds each)

5. Incubate gel in (chilled) 0.1% (w/v) silver solution

• 0.5g silver nitrate in 0.5L water

• Time: 20-40 min, incubate at 4^{o}C

6. Rinse gel with two changes of water (10 seconds each)

7. Develop gel with developing solution (0.04 % formaldehyde in 2 % sodium carbonate)

• 10 g sodium carbonate in 0.5L water

• 540ul formaldehyde (37%) in 0.5L water

• Replace solution when it turns yellow

8. Quench development by discarding the developing solution and replacement with 1% acetic acid when a sufficient degree of staining has been obtained.

9. Store the silver stained gel in 1% acetic acid solution at 4^{o}C (for months).