Silver and Coomassie Stains

Tuesday, April 18, 2017 2:45 PM

Silver Stain

This procedure is for gels that are up to 1 mm thick. Larger gels require larger volumes.

1. Remove the gel from the plastic case and cut off gel foot
2. Place gel in several gel volumes of water and shake for 3-5 min
3. Fix gel: discard water and add **fixing solution**. Shake for 45 min
4. Rinse gel: replace fixing solution with **rinse solution**. Shake for 15 min
5. Wash with water for 10 min; change water and repeat
6. Sensitize gel: replace water with **sensitizer**. Shake for 2 min (maximum time)
7. Wash gel: discard sensitizer and wash 3-5x with water (10 s each)
8. Stain gel: replace water with **cold staining solution**. Shake for 20 min
9. Wash gel 5x with water (10-20 s each) to remove any residual silver from the container
10. Develop gel: after washing, add **developer** and gently swirl the gel. Wait 30 s and dump. Replace with fresh developer. Watch the appearance of the protein bands so you can stop it at the correct time. This should not take more than 5-10 min total.
11. Stop developing: discard developer and add **stop solution**. Shake for 5 to 10 min
12. Wash gel with water (3X)
13. Image in plastic sheet

Solutions--all water (including washes) must be Milli-Q quality

Fixing solution: 50% ethanol, 10% glacial acetic acid
Rinse solution: 50% ethanol
Sensitizer: 0.02% sodium thiosulfate (10 mg for 50 mL solution)
Staining solution: 0.15% silver nitrate (75 mg ultrapure silver nitrate for 50 mL solution), 20 uL formaldehyde (37% v/v)
Developer (50 mL): 4% (2 g) sodium carbonate, 20 uL (of 37% v/v) formaldehyde, 1 mL sensitizer
**you need two of these per gel
Stop solution: 1% acetic acid

Notes:

The first water wash step helps remove any remaining detergents from the electrophoresis step, but you can lose small MW proteins if you do it too long.

The gel stains better if the staining solution starts off cold, so make it during the water wash step and put it in 4C or -20 C for a quick chill.

Be careful not to let the sensitizer step run too long and be sure to wash well after sensitizing. This decreases the yellow background.

If the smaller proteins don't stain well enough even after 2 developer additions, you can stop it, wash the gel in water for 15 min, and then re-stain.

Coomassie Stain

1. Soak the gel in Coomassie solution for 1 hour or until the gel has taken up enough color

2. De-stain it in fixation buffer for at least 1 hour, preferably overnight

Solutions:

Fixation buffer: 45% water, 45% MeOH, 10% acetic acid.
Coomassie blue: 1% coommassie blue in fixation buffer

The Coomassie staining solution can be re-used until you notice your staining efficiency has decreased (i.e. 1 hour no longer stains the gel a dark blue color)