Silver Staining of proteins in a Gel

Stock Solutions

50 % Methanol; 10 % Acetic Acid
10 % Methanol; 5 % Acetic Acid
100 mM DTT
Farmer's Reducer
Solution A - Add 37.5 g potassium ferricyanide and bring up to 500 ml with dH_2O.
Solution B - Add 120 g anhydrous sodium thiosulfate and bring up to 500 ml with dH_2O.

To be prepared fresh (Prepare only what you will use)

0.012 M $AgNO_3$ -- Add 0.041 g $AgNO_3$ to 20 ml dH_2O.

0.28 M Na_2CO_3 + Formaldehyde -- Add 0.6 g Na_2CO_3 and 10µl HCHO to 20 ml dH_2O.

Procedure

1. Fix the gel in 50 % methanol: 10 % Acetic acid for 15 minutes ***(30 minutes)**** or overnight.
2. Wash in 10 % methanol: 5 % Acetic acid for 15 minutes ***(30 minutes)****.
3. Wash 1 minute ***(5 minutes)**** in dH_2O. Repeat 3-5 times
4. Add 2 µl of 100 mM DTT to 100 ml dH_2O add soak for 15 minutes ***(30 minutes)****.
5. Incubate in 0.012 M $AgNO_3$ for 15 minutes ***(30 minutes)****.
6. Wash twice (5 seconds each) in dH_2O.
7. Rinse briefly in approx. 25 ml Na_2CO_3/HCHO.
8. Repeat rinse.
9. Add remaining Na_2CO_3/HCHO and rock gently until bands begin to appear.
10. Remove Na_2CO_3/HCHO when the gel is nearing the desired intensity, and wash repeatedly in large volumes of dH_2O.

Discard all washes & solutions in aqueous waste container NOT IN SINK!!

To Reduce Background Staining

1. Mix 1 ml Farmers Solution A with 4 ml Farmers Solution B and bring up to 100ml with dH_2O.
2. Add the Farmers solution to the gel and watch closely until the gel is nearing the desired destaining intensity.
3. Wash repeatedly in large volumes of dH_2O.
4. Some destaining may occur during the dH_2O washes, so it is best to start the washing a little earlier.

Discard all washes & solutions in aqueous waste container NOT IN SINK!!

* ***Incubation times appearing in parenthesis, bold, and italic are to be used for higher percentage acrylamide gels.***

Silver Staining of proteins on a nitrocellulose blot

OBVIOUSLY NOT FOR USE WITH A BLOT THAT HAS BEEN BLOCKED!!

1. Wash blot in sterile distilled water and leave in water until ready.

2. Make the following solutions:

    a. 40 % Na Citrate
    b. 20 % Silver nitrate
    c. 20 % $FeSO_4 \cdot 7H_2O$ (to be made up fresh every time!!)

3. To a 15 ml tube, add 9 ml water

4. Add 0.5 ml 40 % Na Citrate and mix.

5. Add 0.4 ml 20 % $FeSO_4 \cdot 7H_2O$ and mix.

6. Add 0.1 ml 20 % Silver nitrate and mix very well!! It will form a dark precipitate but this will go back into solution quickly. Use immediately !!!

7. Remove water from blot and quickly add staining solution.

8. Mix vigorously to avoid over staining only parts of the blot.

9. Staining is very rapid so as soon as the bands show up, remove the stain and immediately wash with plenty of running water (place blot directly under stream of water).