**SOP: SP012.1**

## Silver Staining SDS-PAGE Gels

**Materials and Reagents:**

1. 100 ml and 200 ml Erlenmeyer Flasks
2. Pyrex gel staining tray
3. Plastic wrap
4. 1st Fix (see SOP: R002)
5. Periodic acid (optional)
6. 2nd Fix (see SOP: R003)
7. 2% Glutaraldehyde
8. MilliQ $H_2O$
9. Dithiothreitol (DTT)
10. $AgNO_3$ (silver nitrate)
11. $Na_2CO_3$ (sodium carbonate)
12. 37% Formaldehyde
13. 50% Citric acid
14. Shaker table

**Protocol:**

1._____ Run SDS-PAGE gel according to SOP SP007

2._____ Remove the SDS-PAGE gels from the electrophoresis plates, place them in a Pyrex gel staining tray containing enough 1st Fix to cover the gels completely. Cover the staining tray with plastic and place on the shaker table for 45 minutes for small gels and 4 hours for large gels (notes 1 and 2).

3._____ Decant the 1st Fix into the sink.

4._____ **OPTIONAL (for gels containing LAM, LM, or PIM):** Make a 0.7% solution of periodic acid by dissolving 0.7 g of periodic acid in 100 ml of 1st Fix. Add this to the gels. Place the tray back on the shaker, 5 minutes for small gels and 10 minutes for large gels. Decant the periodic acid solution.

5._____ Add enough 2nd Fix to cover the gels and shake, 5 minutes for small gels and 10 minutes for large gels.

6._____ Decant the 2nd Fix into the sink.

7._____ Add enough 2% glutaraldehyde to just cover the gels and shake, 5 minutes for small gels and 10 minutes for large gels (note 3).

8._____ Decant the glutaraldehyde into the glutaraldehyde waste bottle located in the hazardous waste accumulation site.

9._____ Add enough MilliQ $H_2O$ to cover the gels and shake, 10 minutes for small gels and 20 minutes for large gels. Decant off the water into the sink and repeat this step 2 more times, for a total of 3 water washes (note 4).

10._____ Dissolve 5 mg of dithiothreitol in 200 ml of MilliQ $H_2O$. Add this to the gels and shake, 5 minutes for small gels and 10 minutes for large gels.

11._____ Prepare silver nitrate: Dissolve 0.1 g of $AgNO_3$ in 100 ml of MilliQ $H_2O$ (note 5), mix with a stir bar.

12._____ Prepare developer: Dissolve 6 g of $Na_2CO_3$ and 3-6 drops of formaldehyde in 200 ml of MilliQ $H_2O$, mix with a stir bar (note 6).

13._____ Decant off the dithiothreitol solution into the sink. Add the 0.1% $AgNO_3$ solution to the gels and shake, 5 minutes for small gels and 10 minutes for large gels.

14._____ Decant off the silver mixture and rinse the gels 3 times very briefly (approximately 10 seconds each rinse) with MilliQ $H_2O$.

15._____ Pour approximately 1/3 of the developer solution into the gel staining tray, rock by hand and pour off when the liquid starts to turn yellow. Add the rest of the developer to the gels and place on the shaker and allow the gels to develop until the bands are as dark as desired (note 7).

16._____ Once the bands are the desired color, stop the reaction by adding approximately 20 ml of 50% citric acid and place on shaker. Once the bubbling has stopped (15-30 minutes) decant off the citric acid/developer mixture and replace with water.

17._____ Leave the gels in the water until you are ready to scan or dry them (note 8).

18._____ Dry gels and/or scan the gel image.

**Notes:**

1. Small gel refers to the 10 cm x 7 cm x 0.75 mm format, large gel refers to the 20 cm x 20 cm x 1.5 mm format.
2. The gels can be kept in 1st Fix or water (step 9) indefinitely.
3. Glutaraldehyde must be discarded as hazardous waste. In order to reduce the amount of waste accumulation, use a minimum amount of glutaraldehyde (just enough to cover the gels).
4. To reduce the amount of background on the gels, increase the time of each water wash in step 9. An overnight wash works well.
5. There is a small scoop (in the silver staining drawer in C210) that can be used to measure out 0.1 g silver nitrate.
6. The developer solution should be made while the gels are soaking in the dithiothreitol solution (step 10) to allow enough time for the $Na_2CO_3$ to go into solution.
7. It is best to place the staining tray on a paper towel, or other white surface, to give a good background for viewing the gel.
8. Long term storage of gels in water should be in the dark to prevent discoloration of the gel.

**References:**

Morrisey, J.H. 1981. Silver stain for proteins in polyacrylamide gels; a modified procedure with enhanced uniform sensitivity. Anal. Biochem. 117:307-310.