SOP: SP012.3
Modified 6.15.2023 by KCB

Silver Staining SDS-PAGE Gels

Materials and Reagents:
1. 100 ml and 2x 200ml Erlenmeyer Flasks (Note 1)
3. Pyrex glass gel staining tray
4. 1st Fix (see SOP:R002)
5. Periodic acid (optional)
6. 2nd Fix (see SOP: R003)
7. 2% Gluteraldehyde
8. DI 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
15. Plastic cling wrap

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

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

3. _____ **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.

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

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

6. _____ Add enough 2% gluteraldehyde to just cover the gels and shake, 5 minutes for small gels and 10 minutes for large gels.

7. _____ Decant the gluteraldehyde into the gluteraldehyde waste bottle located in the biohazard waste accumulation site.

8. _____ 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 and repeat this step 2 more times, for a total of 3 water washes (note 3).

9. _____ 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.

10. _____ Decant off the dithiothreitol solution into the sink.

11. _____ Make solution of 0.1% $AgNO_3$ by dissolving 0.1 g of $AgNO_3$ in 100 ml of MilliQ H_2O (note 4). Add this to the gels and shake, 5 minutes for small gels and 10 minutes for large gels.

12. _____ Decant off the silver mixture and rinse the gels 3 times very briefly (approximately 30 seconds) with MilliQ H_2O.

13. _____ Make the developer by dissolving 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 5).

14. _____ 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 6).

15. _____ Once the bands are the desired color, stop the reaction by adding 20 ml of 50% citric acid. Once the bubbling has stopped (approximately 30 minutes) replace the citric acid with water.

16. _____ Leave the gels in the water until you are ready to scan or dry them (note 7).

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

Notes:
1. The gels can be kept in 1st Fix and water (step 8) indefinitely.
2. 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.
3. To reduce the amount of background on the gels, increase the time of each water wash in step eight. An overnight wash works well.
4. There is a small scoop (in the silver staining drawer in C210) that can be used to measure out 0.1 g silver nitrate.
5. The sodium carbonate-formaldehyde developer solution should be made while the gels are soaking in the dithiothreitol solution (step 9). This allows enough time for the Na_2CO_3 to go into solution.
6. 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.
7. Long term storage of gels in water should be in the dark to prevent discolorization 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.