**Silver Stain**

* **Required Items**
* Glacial Acetic Acid
* **Fix 1** 
  + 30% Ethanol
  + 10% Acetic Acid
* **Fix 2**
  + 30% Ethanol

To be made fresh each time

* **2.5 mM Sodium Thiosulfate**
  + 0.32 g in 500 mL dH2O
* **Silver Stain**
  + 0.2 g AgNO3
  + 80 μL 37% Formaldehyde
  + 100 mL dH2O
* **Revelation Solution**
  + 3 g Na2CO3
  + 50 μL 37% formaldehyde
  + 800 μL 2.5 mM sodium thiosulfate
  + 100 mL dH2O

1. While wearing gloves, place your gel in a plastic or glass container, and add a volume of **Fix 1** so that it covers it. Place the container on a rocker and incubate for 30 minutes at room temperature. After incubation, pour off Fix I while leaving the gel.
2. Repeat **Fix 1** incubation for an additional 30 minutes (the Fix I steps can be combined in an overnight incubation).
3. Incubate with **Fix 2** for 5 minutes.
4. Repeat step 3 two additional times.
5. After pouring off the final Fix II solution, rehydrate the gel with **dH2O** and incubate with rocking for 20 minutes. Pour off the water
6. Add **2.5 mM Sodium thiosulfate** and incubate with rocking for 2 minutes (sodium thiosulfate helps reduce background).
7. Rinse the gel by adding dH2O, and incubate with rocking for 10 minutes.
8. Repeat step 7 two additional times.
9. After pouring off the water, add **Silver Stain** and incubate with rocking for 30 minutes.
10. Pour off the silver stain into an approved waste container for disposal.
11. Rinse the gel with **dH2O** for 30 seconds (TIME IS CRITICAL), then pour it off.
12. Add **Revelation Solution** and rock by hand until the bands appear with the desired intensity.
13. Stop the reaction by adding **Glacial acetic acid** directly to the revelation solution in the container. After bubbling has subsided, pour off the solution. Take an image of the gel.

**Silver Stain Checklist**

1. Fix 1, 30 minutes \_\_\_\_\_\_\_\_\_\_
2. Fix 1, 30 minutes \_\_\_\_\_\_\_\_\_\_
3. Fix 2, 5 minutes \_\_\_\_\_\_\_\_\_\_
4. Fix 2, 5 minutes \_\_\_\_\_\_\_\_\_\_
5. Fix 2, 5 minutes \_\_\_\_\_\_\_\_\_\_
6. dH2O, 20 minutes \_\_\_\_\_\_\_\_\_\_
7. 2.5 mM Sodium thiosulfate, 2 minutes \_\_\_\_\_\_\_\_\_\_
8. dH2O, 10 minutes \_\_\_\_\_\_\_\_\_\_
9. dH2O, 10 minutes \_\_\_\_\_\_\_\_\_\_
10. dH2O, 10 minutes \_\_\_\_\_\_\_\_\_\_
11. Silver stain, 30 minutes \_\_\_\_\_\_\_\_\_\_
12. dH2O, 30 seconds (time critical)
13. Revelation
14. Stop reaction with Glacial acetic acid