**[Silver Staining Procedure](https://www.bio-rad.com/sites/default/files/webroot/web/pdf/lsr/literature/LIT-442.pdf) (**[**Bio-Rad 1610449**](https://www.bio-rad.com/en-us/sku/1610449-silver-stain-plus-kit?ID=1610449)**)**

**Silver Stain Materials:**

* + Development accelerator – Stored at 4°C (Bio-Rad 1610448)
  + Fixative enhancer (Bio-Rad 1610461)
  + Silver complex (Bio-Rad 1610462)
  + Reduction moderator (Bio-Rad 1610463)
  + Image developer (Bio-Rad 1610464)
  + 5% Acetic acid

**Development Accelerator Reagent Preparation (only if not prepared):**

1. Place 400 ml of deionized distilled water in a 600 mL cylinder containing a PTFE-coated stir bar
2. Begin stirring and slowly add 25 g of development accelerator reagent. Stir until dissolved.
3. Add water to make 500 mL. Store in plastic 1 L bottle labeled development accelerator solution. (Initial and Date – \*\*Reagent is good if used within 3 months\*\*)
4. Store at 4°C. Use at room temperature.

**Silver Stain Methods:**

1. Remove development accelerator solution from fridge, measure out 50 mL and let it warm to room temperature prior to use
2. Prepare Fixative enhancer solution:

|  |  |  |
| --- | --- | --- |
| (50%) - 50.0 | mL | Methanol |
| (10%) - 10.0 | mL | Acetic Acid |
| (10%) - 10.0 | mL | Fixative Enhancer Concentrate |
| (30%) - 30.0 | mL | Water |

1. Place each dried out in a container with 50mL of the fixative enhancer solution.
2. Rock gentle on rocker to fix the gels for **30 minutes**.
3. Placing on finger on the gel to hold in place, pour out the fixative enhancer solution
4. Dump deionized distilled water into the container, shake for a couple seconds and pour out the water
5. Rock the gels gentle on rocker in 200 mL deionized distilled water for **20 minutes**
6. Placing on finger on the gel to hold in place, pour out the deionized distilled water
7. Dump the deionized distilled water into the container, shake for a couple seconds and pour out water
8. Rock the gels gentle on rocker in 200 mL deionized distilled water for an additional **20 minutes.**
9. After **15 minutes**, prepare the staining solution by placing 35 mL deionized water into a 150 mL beaker and stir with a PTFE-coated stir bar.
10. Add 5.0 ml Silver Complex Solution to the beaker, allow to stir for 5 seconds
11. Add 5.0 ml Reduction Moderator Solution to the beaker, allow to stir for 5 seconds
12. Add 5.0 ml Image Development Reagent to the beaker, allow to stir for 5 seconds
13. After the gels have rocker in deionized distilled water for an additional 20 minutes, quickly add 50 ml of the room temperature development accelerator solution to the beaker of staining solution. Swirl well.
14. Quickly dump out the deionized distilled water and 50 ml of the staining solution to each gel and gently rock.
15. Set a timer for 20 minutes so you can track how long the gels have been in staining solution. Staining time will favor but pay close attention from 5 minutes onward.
    1. Note: Staining time is dependent on the sample and the quantity loaded.
16. During the first 5 minutes of staining make sure you have 200 mL of 5% acetic acid ready.
17. Once desired staining intensity is reached, dump the stain in bottle under fume hood labeled “Silver Stain” and pour in 50 mL of 5% acetic acid to stop the reaction and rock for **15 minutes**
18. Dump the acetic acid and pour second 50 mL of 5% acetic acid into the containers and rock until you are ready to image.

**Silver Stain Tips:**

* To prevent silver deposits on staining trays and inconsistent staining, all containers used for mixing and staining should be scrupulously clean. Glass, polyethylene, or polypropylene containers may be cleaned with 50% (approx. 8 N) nitric acid after cleaning with laboratory detergent. Rinse thoroughly with high-quality deionized water
* Throughout the procedure the gel should always be completely submerged in solution. Gels that float on the surface of the solution will not stain consistently and will show background discoloration
* Avoid staining gels in direct sunlight or at temperatures above 25°C
* Never touch gels with metal objects or bare skin. PVC or latex gloves rinsed with deionized water should be used if the gel must be handled. Perform gel manipulations with glass or polyethylene rods, if possible

Stopping point: The fixative step is a desirable stopping point if there is not enough time to complete the procedure