

Silver Stain for Acrylamide Gels

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Introduction

Silver staining is a redox-based staining method for proteins on electrophoresis gels. We use this method to assess interactions between salivary protein and tannin after native gel electrophoresis, or to assess composition of crude saliva after SDS gel electrophoresis.

This method is more sensitive than Coomassie staining, but is also more expensive. Proteins may be stained either as dark bands on the gel or as light bands against a faint background. Tannin enhances the overall sensitivity of the stain. However, tannin also reacts with the stain to give dark brown smears which can interfere with interpretation of the gel. The fixing step described here minimizes this interference, and also ensures that proline-rich proteins are properly fixed in the gel.

The method given here is modified from Hochstrasser, Patchornik, and Merril, *Anal. Biochem.* 173: 412-423 (1988). These amounts and times are for small, 0.75 mm thick gels. For larger gels you would need larger volumes of the same solutions; for thicker gels you would need longer times to allow complete diffusion of the solutions into the gel.

Reagents

- 6% perchloric acid: 86 mL of reagent (70%) perchloric acid, HClO₄, up to 1 liter with water. Perchlorate salts are contact explosives, and perchloric acid is a strong oxidizing acid. Be careful.
- Fix: Prepare a stock solution of 257 mL ethanol plus 600 mL water. Just before use, mix 130 mL of the stock with 22 mL reagent (37%) formaldehyde. If the formaldehyde solution is cloudy, do not use it--it will make it impossible to properly develop the stain later.
- SDS wash: 200 mL ethanol, 100 mL glacial acetic acid, 1700 mL water. (Used to remove SDS from SDS gels).
- Stain: Prepare 20% silver nitrate just before use (4 g AgNO₃ up to 8 mL distilled water; chloride in the water will give you a cloudy solution that cannot be used). Just before staining, mix 1.5 mL concentrated ammonia, 200 μ L of 10 N NaOH, and 20 mL distilled water. Then add, dropwise with constant stirring, the silver nitrate solution. As you add the silver, masses of brown precipitate will

form (silver hydroxide) and then disappear (ammoniacal silver). The solution should be clear and colorless when you are done. Then bring the final volume to 100 mL with distilled water and mix.

- Developer: Prepare a stock solution of citric acid (4 g citric acid up to 200 mL with distilled water). This must be refrigerated because microorganisms love to grow in citric acid solutions. Just before using, mix 100 mL water, 500 uL citric acid stock, and 100 uL reagent formaldehyde (37%).
- Stop: 1 mL glacial acetic up to 100 mL with water.

Method

1. Acid fix the gel for exactly 10 min in the perchloric acid solution. Agitate while fixing.
2. Wash the gel for exactly 5 min with distilled water. Agitate.
3. Fix the gel overnight in the ethanol/water/formaldehyde fix solution.
4. At this point, I transfer the gels into individual plastic trays--they sometimes stick to glass trays in the next steps, which causes poor staining or tearing.
5. Wash SDS gels 4 times, 10 min each wash, with the SDS wash solution, agitating while washing. Skip this step for native gels.
6. Wash with water 3 times, 10 min each wash, then 1 time 30 min. Agitate. You can be pretty flexible on the times in this step, but be sure to wash long enough.
7. Stain for 10 min with agitation. At this point, some brown streaks (tannin) may appear on the gels, but bands of protein will not be visible yet.
8. Wash 3 times with water, 5 min each time, with agitation.
9. Develop, on a light box to monitor bands. Stop when development is satisfactory. Store gels temporarily in stop, then transfer to water and dry between sheets of cellophane.