Bio-Rad Silver Stain

Catalog Numbers
161-0443
161-0444
161-0445
161-0447

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Section 1
Introduction

Silver staining is a highly sensitive method for detecting proteins and nucleic acids in polyacrylamide slab gels. The Bio-Rad Silver Stain, derived from the method of Merril,1 is 10-50 fold more sensitive than Coomassie brilliant blue R-250 for proteins (detection is ~0.1 ng/mm2) and 2-5 fold more sensitive than ethidium bromide for single and double stranded DNA and RNA. Approximately 24 full size gels or 48 mini gels can be stained with one kit.

Section 2
Silver Stain Kit Components

Oxidizer Concentrate - A 10-fold stock solution. Contains potassium dichromate (CAS 7778-50-9) and nitric acid. Store at 4 ˚C.

Warning: Strong oxidizer - avoid contact with reducing agents.

Irritant - avoid contact with skin and eyes. Wear gloves and eye protection.

Silver Reagent Concentrate - a 10-fold stock solution. Contains silver nitrate (CAS 7761-88-8). This solution is heat sensitive. Store at 4 ˚C.

Warning: Poisonous - caustic to eyes, skin, and mucous membranes. Wear gloves and eye protection.

Developer - Four packages of dry chemical blend. Contains sodium carbonate and paraformaldehyde (CAS 30525-89-4). Store at 4 ˚C.

Warning: Poisonous

Irritant - vapor and dust irritates eyes, mucous membranes, and skin. Wear gloves, lab coat, and eye protection. Fume hood recommended.

Section 3
Important Procedural Information

Read the entire protocol, procedural information, and the Troubleshooting Guide before beginning.

3.1 Water Purity

Deionized water of less than 1 µmho conductivity is recommended for all phases of the procedure. Contaminants such as chloride ions will precipitate silver ions causing reduced sensitivity and increased background. Water suspected of containing interfering ions can be thoroughly deionized by passage over a column of AG® 501-X8(D) mixed bed ion exchange resin (catalog number 142-6452).

3.2 Gel Handling

Wear rubber gloves that have been washed and rinsed with deionized water. Use glass containers and make sure that the volumes are sufficient to allow free movement of the gel during shaking. An orbital shaker is recommended to promote mixing during all steps. Use a separate dish for each gel. Do not put pressure on gels during handling or when decanting solutions.

3.3 Temperature

The oxidizer, silver reagent, and developer solutions should be at room temperature (23-25 ˚C) for use. If heating is necessary, dilute the oxidizer and silver reagent before warming to 25 ˚C. Use immediately. The developer can be heated to 50 ˚C to enhance development (see "Band Development" below).

3.4 Convenient Stopping Point

Protein gels can be stored indefinitely in 40% methanol/10% acetic acid prior to staining.

3.5 Band Development

Bands usually appear dark brown against a pale background. The duration of the development steps is very approximate, and development should be monitored closely. The third volume of developer may not be necessary, especially when a water wash is used between the silver reagent and developer steps.

Stop development when the bands reach the desired intensity in relation to background. If drying the gel on filter paper, stopping development just before the desired intensity is reached will help keep the gel from turning darker on the filter paper (see Troubleshooting Guide). The rate of development is highly temperature dependent. Developer can be heated to 50 ˚C if faster development is desired.

3.6 Destaining

Gels allowed to develop too long will have high background or surface deposits of silver (mirroring). Destaining can be performed using a photographic reducer such as the following, which was developed by Switzer, et al.[3]

Dissolve 37 g of sodium chloride and 37 g of cupric sulfate anhydrous in 850 ml of deionized water. Add concentrated ammonium hydroxide until the precipitate that forms is completely dissolved to give a deep blue solution. Adjust to 1 liter with deionized water. Prepare a second solution containing 436 g sodium thiosulfate pentahydrate in 1 liter deionized water. Prepare the destain by combining equal parts of the two solutions immediately prior to use. Remove the gel from the destain before complete destaining has occurred. Wash several times in deionized water to remove the destain and prevent fogging. Stop the destaining process with 10% acetic acid for 15 minutes. Wash the gel for at least 1 hour with several changes of water. To restain, begin with step 8, Table 1.

An alternative destain is Kodak rapid fix, solution A. Add 12 ml of rapid fix to 88 ml deionized water to prepare the destain. Stop the destaining process with 2.4 g Kodak hypo clearing agent dissolved in 100 ml deionized water, for 15 minutes. Wash the gel for at least 1 hour with several changes of water. To restain, begin with step 8, Table 1.

3.7 Storage of Stained Gels

After staining is complete, gels can be stored indefinitely in zip-lock plastic bags with just a few drops of water. Alternatively, gels may be dried on filter paper. Change the stop solution two or three times (at least 5 minutes for each step) to remove all of the developer before drying. This will prevent continued development. Drying gels between two pieces of cellophane also eliminates the problem of the gel darkening upon drying.

3.8 Photographing Gels

Gels may be photographed during development or after stopping with 5% acetic acid. Photographing should be done on a bright light box, however, development may be accelerated if it has not yet been stopped. If the staining container is not optically clear, the gel should be placed on a glass plate or directly on the light box for photographing.

Suggested Settings

Camera	Polaroid-type (Polaroid Model MP-4)	35 mm (50 mm lens)
Film	Type 667, positive	Kodak panatomic-X, asa 32
F stop	F32	F16
Speed	1/125 sec.	automatic setting
Height	1-2 feet	1-2 feet (on tripod)

Section 4
Reagent Preparation

Prepare the oxidizer and silver reagent solutions on the same day that staining is to be performed:

	Full Size Gels			Mini Gels		
	ml Reagent	ml H_2O	Total ml	ml Reagent	ml H_2O	Total ml
Oxidizer	20	180	200	10	90	100
Silver Reagent	20	180	200	10	90	100

Developer Dissolve one bottle in 3.6 liters of deionized water by stirring for 15 minutes at room temperature. This is enough for 6 full sized gels or 12 mini gels. To prepare smaller amounts, use 32 grams of developer per liter of deionized water. Be sure to shake the bottle thoroughly before weighing out, since the components will settle out and separate. Failure to mix the contents can result in very slow or no development. Store the solution at 23-25 ˚C for up to 1 month. Keep the solution tightly covered to avoid evaporation of paraformaldehyde.

Section 5
Protocols

Table 1. Silver Stain Protocol

Begin with step 1 immediately after the electrophoretic run is complete and follow through to step 13 as indicated. For a convenient stopping point, gels can be stored in 40% methanol/10% acetic acid (step 1) indefinitely.

Reagent	Volume*	Duration <0.5 mm gel	0.5-1.0 mm gel	>1.0 mm gel
1. **Fixative** 40% methanol/10% acetic acid (v/v)	400 ml	30 min	30 min	60 min
2. **Fixative** 10% ethanol/5% acetic acid (v/v)	400 ml	15 min	15 min	30 min
3. **Fixative** 10% ethanol/5% acetic acid (v/v)	400 ml	15 min	15 min	30 min
4. **Oxidizer**	200 ml	3 min	5 min	10 min
5. **Deionized water**	400 ml	2 min	5 min	10 min
6. **Deionized water**	400 ml	2 min	5 min	10 min
7. **Deionized water**	400 ml	—	—	10 min
Repeat washes 5, 6, 7 until all the yello color is removed from the gel.				
8. **Silver reagent**	200 ml	15 min	20 min	30 min
9. **Deionized water**	400 ml	—	1 min	2 min
10. **Developer**	200 ml	~30 sec. Develop until solution turns yellow or until brown “smokey” precipitate appears. Then pour off developer, and add fresh developer.		
11. **Developer**	200 ml	~5 min	~5 min	~5 min
12. **Developer**	200 ml	—	~5 min	~5 min
13. **Stop** 5% acetic acid (v/v)	400 ml	~5 min	~5 min	~5 min

* Recommended volumes for a 16 cm slab gel in a standard 21 x 21 x 5 cm baking dish. For smaller gels in a smaller container, volumes may be lowered proportionally. For example, for a mini vertical gel (8 x 7 cm) you can decrease all volumes by one-half. The glass container should be of appropriate size to allow free movement of the gel during shaking while maintaining a volume of reagent sufficient to cover the gel completely.

Table 2. Modified Silver Stain Protocol

This modified protocol, optimized for mini gels (~7 cm x 8 cm x 0.75 mm), gives clear backgrounds and consistent results in less time than the standard protocol. Incubation times may need to be increased for large format gels.

	Reagent	Duration
1.	**Fixative:** 40% methanol/10% acetic acid (v/v)	30 minutes minimum. Gel may be stored overnight at this step.
2.	**Oxidizer:** *Note: make sure gel is completely immersed.*	5 minutes
3.	**Water Washes:** use large volumes of water and change the wash many times (6-7), especially in the first 5 minutes. This flushes the oxidizer from the gel without removing it from the proteins. Proceed to step 4 after 15 minutes even if gel is still slightly yellow in color.	15 minutes maximum
4.	**Silver Reagent:**	20 minutes
5.	**Quick Water Rinse:**	30 seconds maximum
6.	**Developer:**	~ 30 seconds or until a brown or smokey precipitate appears. Quickly pour off the solution and add fresh developer. Repeat this step if precipitate appears again. If the solution remains clear, the gel can remain in developer for about 5 minutes. Change developer every 5 minutes until desired intensity is obtained.
7.	**Stop:** 5% acetic acid (v/v) *Note: make sure gel is completely immersed.*	15 minutes

Section 6
Additional Applications

6.1 Nucleic acid staining

Follow the silver staining protocol, deleting the acetic acid from the fixatives (steps 1, 2, and 3, Table 1). Double and single stranded DNA and RNA in polyacrylamide gels can be stained. Silver staining is not recommended for agarose gels.

6.2 Silver staining after Coomassie Brilliant Blue R-250 staining

Completely destain the Coomassie stained gel in 40% methanol/10% acetic acid. Begin at step 2 of the silver staining protocol and proceed as usual.

6.3 Silver staining electrofocusing slab gels

Fix the gel in 30% methanol/10% trichloroacetic acid/3.5% sulfosalicylic acid for 1 hour. Follow by at least 2 hours in several volumes of 30% methanol/12% trichloroacetic acid to insure complete removal of ampholytes. Go to step 2 of the protocol and proceed as indicated. Store stained gels in 40-50% methanol (other low % gels may also be stored this way to control swelling). **Modifications for gels bonded to a polyester backing:** If gel is < 0.5 mm thick, modify wash steps 5 through 7 to 2 x 5 minutes in 400 ml deionized water. Increase step 9 to 1 minute in 400 ml deionized water. For gels > 0.5 mm thick, modify steps 5 through 7 to 2 x 10 minutes and increase step 9 to 1 minute in 400 ml deionized water.

Section 7
Troubleshooting Guide

Problem	Solution
Gray or brown precipitate appearing as smudges or swirling on gel surface. May become mirror-like. Bands may be faint or absent.	Non-specific deposition of silver due to oxidizer or silver reagent carry-over. Increase wash steps: **gels ≤ 1 mm:** Increase steps 5, 6, and 7(including any omitted steps) to 5 minutes each. Increase step 9 to 2 minutes. **gels >1 mm:** Increase steps 5, 6, and 7 to 20 minutes each.

Troubleshooting Guide (continued)

Problem	Solution
	Temperature too low. Make sure temperature of all silver stain reagents is at least 23 ˚C.
	Mirroring can be caused by the developer precipitate sticking to the gel surface. Be sure to decant first development solution as soon as the precipitate appears (see protocol).
Dark uniform background, usually yellow.	Oxidizer is not completely removed. Increase wash steps 5, 6, and 7 as described above, removing all traces of yellow, before going on to silver reagent step.
Mottled background, usually brown or green, with poor sensitivity.	Contaminants in water. Check that the conductivity of the water is less than 1 µmho. See "Notes on Silver Staining."
	Incomplete removal of gel buffer components. Increase times of steps 1, 2, 3 to insure complete removal.
Slow or no development.	Rate of development is highly temperature dependent. Developer solution can be heated to 50 ˚C to speed development.
	Developer solution is too old (paraformaldehyde has evaporated). Shelf life of solution is 1 month at 23-25 ˚C.
	Settling and separation of the components of the developer powder will occur. When preparing solutions of less than 3.6 liters, mix the contents thoroughly before aliquoting.
Gel continues to develop or becomes darker upon drying onto filter paper.	Increase stop time (step 13) and change stop solution 2 or 3 times to remove all developer. Dry between two pieces of clear cellophane or photograph before drying onto filter paper.

Problem	Solution
Some proteins are not staining.	Silver stain as usual, then recycle gel. Soak in deionized water for 30 minutes, then repeat the silver staining starting with the silver reagent step (step 8). Proteins that did not stain on the first cycle will stain to full intensity
Negative staining occurring.	Proteins are overloaded. Recycle gel as above. Some metalloproteins will always stain negatively.
	Silver reagent is being washed away. Decrease time of wash in step 9.
Contaminant bands across entire gel, occurring at 58 kD and 64 kD.	Skin keratin (from dandruff, ungloved hands) contaminating solutions. Wear gloves at all times during the pouring and running of gels and solution preparation. Filter all solutions through a 0.45 µm nitrocellulose.
Large discolored spots on gel.	Any pressure on gel will cause the gel to stain darker at that spot. Avoid crushing the gel with fingers or dish when decanting solutions or transferring gel to a new solution.

Section 8
Ordering Information

Catalog Number	Product Description
161-0443	**Silver Stain Kit,** includes 1 bottle oxidizer concentrate, 1 bottle silver reagent concentrate, and 4 bottles developer. Enough to stain approximately 24 16 cm slab gels.
161-0444	**Oxidizer Concentrate**, 480 ml
161-0445	**Silver Reagent Concentrate**, 480 ml
161-0447	**Silver Stain Developer**, 4 x 115 g

Section 9
References

9.1 General

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9.4 Isoelectric Focusing

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