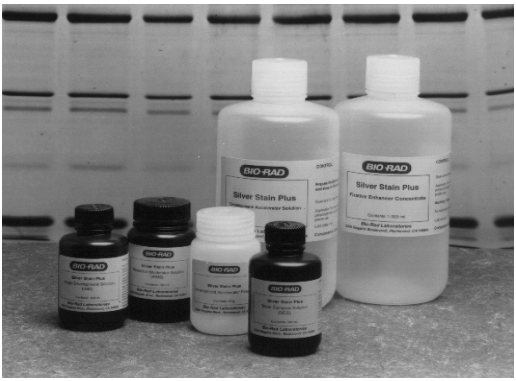
Silver Stain Plus™

Catalog Number
#161-0449



For Technical Service, call your local Bio-Rad office or
in the U.S. call 1-800-424-6723

BIO-RAD

Introduction

Silver Stain Plus™ is a quick, simple system for detecting proteins or nucleic acids in polyacrylamide and dried agarose gels after electrophoresis. Proteins and nucleic acids can be visualized in 1 hour with very little hands-on time by employing a carrier-complex silver staining chemistry similar to that developed by Gottlieb and Chavko for detecting DNA in agarose gels.1 Silver staining is very sensitive. Silver Stain Plus is 30 to 50-fold more sensitive than Coomassie Blue R-250 dye and will detect nanogram quantities of protein and DNA. To ensure best results, always use high-quality deionized distilled water and extremely clean glassware. Always wear gloves when using the kit components and when handling gels.

Components

The Silver Stain Plus kit includes the following components in quantities sufficient to stain 40 mini gels (8 x 10 cm) or 13 conventional gels (16 x 16–20 cm):

- Fixative enhancer concentrate
- Silver complex solution (contains NH_4NO_3 and $AgNO_3$)
- Reduction moderator solution (contains tungstosilicic acid)
- Image development reagent (contains formaldehyde)
- Development accelerator reagent (contains Na_2CO_3)
- Empty 1 L bottle for development accelerator

Additional Materials Needed

In addition to the components in the Silver Stain Plus kit, the following materials are required:

- Reagent-grade methanol and acetic acid
- Deionized, distilled, or high-purity water
- Water of 18-megohm-cm resistivity recommended
- Polytetrafluoroethylene (PTFE)-coated stir bars
- Glass or plastic staining trays

All steps in this procedure require gentle agitation of the gel in solution. For best results, a shaker table is recommended.

Warnings and Precautions

Read each label in the kit before beginning the procedure.

Wear gloves, safety glasses, and protective clothing while preparing and working with all the solutions in this kit.

The image development reagent should be used only in areas with good ventilation. Avoid breathing vapors. Avoid contact with skin. In case of contact with eyes, flush with copious amounts of water and contact a physician.

See Material Safety Data Sheets for additional information.

Storage

All reagents except the development accelerator solution can be stored at room temperature. The development accelerator solution should be stored at 4°C but allowed to reach ambient temperature before use. This may be done by aliquoting the volume needed and either warming in a water bath for 2–5 min or allowing to stand at room temperature for approximately 30 min before use.

Recommendations for Optimal Staining

- 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

Reagent Preparation
Development Accelerator Solution

1. Prepare once for use with the entire contents of the kit.
2. Place 950 ml of deionized distilled water in a 1 L cylinder containing a PTFE-coated stir bar.
3. Begin stirring and slowly add the entire 50 g contents of the development accelerator reagent. Stir until dissolved.
4. Add water to make 1 L. Pour the solution into the provided empty 1 L bottle labeled development accelerator solution.
5. Store at 4°C. Use at room temperature.

If the entire solution will not be used within 3 months, prepare the development accelerator solution according to your use rate.

To prepare half the solution, add 25 g development accelerator reagent to 475 ml deionized distilled water, add water to make 500 ml.

Polyacrylamide Gel Staining Procedure

The following preparations are adequate for staining two mini gels (8 x 10 cm), 0.75–1.0 mm thick. Refer to Table A for staining gels of other sizes.

1. Fixative Step — 20 min

Fixative enhancer solution preparation (for 2 mini gels)

Reagent-Grade Methanol	200 ml	50% V/V
Reagent-Grade Acetic Acid	40 ml	10% V/V
Fixative Enhancer Concentrate	40 ml	10% V/V
Deionized Distilled Water	120 ml	30% V/V
Total	400 ml	100% V/V

After gel electrophoresis, place gels in the fixative enhancer solution. With gentle agitation fix the gels for 20 min. Refer to Table A for fixing times and solution volumes for larger gels.

2. Rinse Step — 20 min

Decant the fixative enhancer solution from the staining vessel. Rinse the gels in 400 ml deionized distilled water for 10 min with gentle agitation. After 10 min, decant water and replace with fresh rinse water. Rinse for an additional 10 min. Decant rinse water. For larger and thicker gels, rinse with 800 ml water for 40 min.

3. Staining and Developing Step — 20 min

Staining Solution Preparation and Procedure (prepare within 5 min of use)

Place 35 ml deionized water into a large beaker or Erlenmeyer flask and stir with a PTFE-coated stir bar. Add the following to the beaker in this order:

5.0 ml Silver Complex Solution

5.0 ml Reduction Moderator Solution

5.0 ml Image Development Reagent

Immediately before use quickly add 50 ml of the room temperature development accelerator solution to the beaker. Swirl well. Add the contents of the beaker to the staining vessel. Stain with gentle agitation.

Stain both mini and large-format gels for approximately 20 min or until desired staining intensity is reached. It may take at least 15 min before the bands first become visible. Note: Staining time is dependent on the sample and the quantity loaded. After the desired staining is reached place the gels in 5% acetic acid to stop the reaction.

4. Stop Step — 15 min

Prepare a 5% acetic acid solution to stop the staining reaction.

Place gels in stop solution for a minimum of 15 min. After stopping the reaction rinse the gels in high-purity water for 5 min. The gels are then ready to be dried or photographed.

Table A. Times and volumes required to stain 2 gels.

Step	Gel Thickness 0.75 to 1.0 mm			Gel Thickness 1.5 to 3 mm		
	Time	Mini Gel	Large Gel	Time	Mini Gel	Large Gel
Fixative	20 min	400 ml	800 ml	30 min	400 ml	800 ml
Water wash	10 min	400 ml	800 ml	20 min	400 ml	800 ml
Water wash	10 min	400 ml	800 ml	20 min	400 ml	800 ml
Stain	20 min	100 ml	300 ml	20 min	100 ml	300 ml
Stop	15 min	400 ml	400 ml	15 min	400 ml	400 ml

Agarose Gel Staining

To prepare an agarose gel for silver staining, it is first necessary to dry the gel after electrophoresis. When the gel is dry it will be clear, wafer-thin, and easy to handle. Follow any one of the three following techniques for drying agarose gels up to 6 mm thick. When the gel is dried, follow the procedure for polyacrylamide gel staining.

Suggested Techniques for Drying Agarose Gels

Air Drying

Place the gel on the hydrophobic side of a sheet of gel support film and air dry in a hood overnight. If necessary, use tape to keep the gel in place. Remove the gel from the support film and follow the procedure for polyacrylamide gel staining.

Compression Drying

Sandwich the gel between two pieces of dry cellophane. Place the sandwiched gel on three sheets of filter paper and cover with three sheets of filter paper. Apply a 1–2 kg weight on top of the filter paper. Let dry overnight. When dry, remove cellophane from the gel and follow the procedure for polyacrylamide gel staining.

Vacuum Drying

A gel dryer, such as Bio-Rad's Model 583 gel dryer, is used in this method. First place a sheet of filter paper on the porous gel support plate. Place a sheet of dry cellophane on the filter paper. Lay the gel on top of the cellophane and cover it with a second sheet of cellophane, plastic wrap, or gel support film.

Place the sealing gasket over the gel sandwich and apply vacuum. Do not apply heat. High heat will melt the gel. Dry overnight. When dry, remove the cellophane from the gel and follow the procedure for polyacrylamide gel staining.

Silver Reagent Disposal

The final staining solution is considered hazardous waste and should not be poured down the sink. Place the staining solution in a disposable plastic or hazardous waste container under the hood. In 1–2 days the silver metal will precipitate from the solution. Once the silver has precipitated the aqueous waste can then be decanted from the silver and disposed of separately. Disposal of both the solid and aqueous waste should be according to local and state regulations.

Silver Staining after Coomassie Blue R-250

Completely destain the Coomassie stained gel in 40% methanol/10% acetic acid. Follow with the wash step of the Silver Stain Plus procedure and thoroughly rinse the destain from the gel. Proceed with the staining step.

Destaining

Gels stained with Silver Stain Plus can be completely destained in 1% hydrogen peroxide. Use several changes of 100 ml of 1% hydrogen peroxide per mini gel for 2–15 min or until completely destained. To restain, place gel in fixative enhancer solution (step 1) or 10% acetic acid. Water wash (step 2) and proceed with staining.

Silver Staining Tips

- Containers must be scrupulously clean. Clean glassware and staining containers prevent inconsistencies in staining. Glass, polyethylene, or polypropylene staining vessels may be cleaned with 50% nitric acid after cleaning with laboratory detergent. Rinse thoroughly with high-quality deionized water
- Never touch gels with metal objects or with 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
- If the gel is squeezed, bent, or torn, uneven background staining may result in the affected portion of the gel
- The gel should always be completely submerged in solution. Gels that float on the surface of the solutions will not stain consistently and will show background discoloration

Troubleshooting Guide

Problem	Cause	Solution
The silver precipitates on the staining containers.	Staining containers may contain residues from soap or prior staining solutions, which silver will bind with to form metallic silver.	Silver staining techniques require containers to be thoroughly cleaned before use. After washing containers with laboratory soap and water, rinse with 50% nitric acid solution to remove residues. Water rinse the acid from the container. Use deionized distilled water as a final rinse.
The staining solution is cloudy or contains a precipitate. Note: The presence of a precipitate does not affect the quality of staining but may require longer staining times.	The development accelerator solution is too dilute.	Add 2–10 ml of development accelerator solution until the precipitate disappears. If the precipitate does not disappear, the solution may still be used, but precipitate should be prevented by following these tips. 1. The development accelerator solution must be used at ambient temperature. Precipitate will form if the solution is used cold. 2. Always add the aliquoted volume of development accelerator solution all at once and never a few ml at a time.

Troubleshooting Guide (continued)

Problem	Cause	Solution
Bands are taking longer than 20 min to develop and a high background is developing with the extended staining time.	Since all proteins are not identical longer fixative times may be required in some cases.	Extend the fix step 15–30 min to improve detection. **Note**: The fixative step is a desirable stopping point if the staining procedure cannot be completed in one day.
	The presence of acetic acid still in the gel from the fixative step will slow detection.	Extend the wash times.
	The development accelerator solution was cold when used.	Allow the development accelerator solution to come to ambient temperature before use.

If you have further question about the procedure, call technical service in the U.S. at 1-800-424-6723 or contact your local Bio-Rad office.

Reference

Gottlieb M and Chavko M (1987). Silver staining of native and denatured eucaryotic DNA in agarose gels. Anal Biochem 165, 33-37.

Ordering Information

Catalog Number	Product Description
161-0449	**Silver Stain Plus Kit**, includes fixative enhancer concentrate, silver complex solution, reduction moderator solution, image development reagent, development accelerator reagent, and complete instructions.
161-0448	**Development Accelerator Concentrate**
161-0461	**Fixative Enhancer Concentrate**
161-0462	**Silver Complex Solution**
161-0463	**Reduction Moderator Solution**
161-0464	**Image Development Reagent**

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800*
Austria *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 5044 5699*
Canada *905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *420 241 430 532* ***Denmark*** *44 52 10 00*
Finland *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0*
Greece *30 210 9532 220* ***Hong Kong*** *852 2789 3300*
Hungary *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460*
Mexico *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30*
Poland *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3188*
South Africa *27 861 246 723* ***Spain*** *34 91 590 5200*
Sweden *08 555 12700* ***Switzerland*** *026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88*
United Kingdom *020 8328 2000*

Lit-442 Rev E US/EG *Sig 1212*