Polysciences, Inc.

Corporate Headquarters
400 Valley Road
Warrington, PA 18976
1-800-523-2575
FAX 1-800-343-3291
Email: info@polysciences.com
www.polysciences.com

Europe - Germany
Polysciences Europe GmbH
Handelsstr. 3
D-69214 Eppelheim, Germany
(49) 6221-765767
FAX (49) 6221-764620
Email: info@polysciences.de

Instructions for the ElectroPure™ Silver Stain Kit

Introduction:

Silver staining of proteins in polyacrylamide gels is a highly sensitive technique for visualizing nanogram amounts of proteins. The ElectroPure Silver Stain Kit is based on a simplified silver staining procedure reported by Wray, et al.[1] This method initiates the silver staining of proteins in an alkaline environment, as opposed to staining in a weakly acidic environments[2] where only some proteins may stain. Proteins enriched in glutamic and aspartic acids silver stain well in acidic environment, but basic proteins do not stain as well.[2] In an alkaline environment, silver complexes with the amino groups of proteins and via cysteine and methionine sulfur also takes place.[3] This produces a more even distribution of signal across all proteins.

The kit consists of five solutions: three utilized to prepare a "staining" solution and two for the preparation of a "developing" solution. For optimal performance, follow the protocol and troubleshooting guide carefully. For additional help, please contact the Technical Services Department.

Notes:

- **Use only highly purified water of at least 18 megaohm/cm resistance.**
- **Use only thoroughly cleaned glassware for all steps to ensure the least contamination.**
- **All steps must be done with constant gentle agitation (40-60rpm).**
- **This procedure is intended for 1.0mm mini-gels. For gels of different thicknesses, incubation times may vary. For 1.5mm thick gels, double all incubation times.**

Procedure:

1. After electrophoresis, rinse the gel for 10-15 seconds with deionized water in the container that will be used for the entire procedure. Before placing the gel in the container, make sure there is deionized water in the container to prevent the gel from sticking to the container.

2. Soak the gel in 100ml fixative solution containing 50% reagent grade methanol and 0.1ml of Solution E (37% formaldehyde) for at least 30 minutes. The gel can be soaked in fixative for up to 3 days, although this may affect contrast. See the Helpful Hints section for further details concerning gel fixation.

3. Prepare the Staining Solution (composed of Solutions A, B, and C):
   Place 100ml of deionized water in a beaker.
   Add the following dropwise while stirring or vortexing:
   1.0ml Solution A (contains sodium hydroxide)
   1.4ml Solution B (contains ammonium hydroxide)
   1.0ml Solution C (contains silver nitrate)

4. Pour off the fixative and briefly rinse the gel and container walls with deionized water (10-15 seconds). Add the staining solution. Soak the gel in the Staining Solution for 15 minutes with constant gentle agitation. Be sure the gel is totally submerged in the solution.

5. Pour off the Staining Solution into a silver waste container (see Helpful Hints). Rinse the gel and walls of the container thoroughly with deionized water. Then, allow the gel to soak in two 5 minute washes of deionized water.

6. Prepare the Developing Solution* (composed of Solutions D and E):
   Place 250ml of deionized water in a container.
   Add and mix:
   1.0ml Solution D (contains citric acid)
   0.25ml Solution E (37% Formaldehyde)

   ***Use this solution within 2 hours after preparation**.

7. Prepare a Stop Solution:
   45% Methanol (reagent grade)
   1-3% Glacial Acetic Acid

8. Soak the gel in the 250ml of the Developing Solution until the bands appear. The bands should appear in less than 15 minutes.

9. When the stain intensity reaches the desired level, quickly pour off the Developing Solution and add the Stop Solution to halt stain development. Consult the Trouble Shooting Guide if there are any problems.

10. Allow the gel to soak 30 minutes to 1 hour in Stop Solution. Wash the gel twice for about 15 minutes in 30% - 50% methanol to prevent fading.

11. The gel is ready for storage. See the Helpful Hints section for appropriate storage methods.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

© Polysciences, Inc. 0904 RevB

Handling Precautions:

Solutions A and B: DANGER! These solutions are corrosive. They can cause burns. Do not get the solutions in eyes, on skin, or on clothing. Avoid breathing dust, vapor, or mist. Keep container closed. Use with adequate ventilation. Wash thoroughly after handling.

First Aid: In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Call a physician. Wash clothing before reuse. Discard contaminated shoes.

Solution C: Oxidizing Substance. Avoid contact with oxidizable materials. Avoid contact with skin and eyes.

First Aid: In case of contact, flush skin or eyes with plenty of water for at least 15 minutes. For eye or skin irritation, consult a physician.

Solution D: Store at room temperature.

Solution E: Caution - This solution contains formaldehyde. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Avoid breathing vapors. Use only with adequate ventilation. Keep container closed.

First Aid: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician. Flush skin with water. Wash clothing before reuse.

NOTE: The full chemical, physical, and toxicological properties of all these solutions are not known. Avoid contact with skin, eyes, or respiratory system.

Helpful Hints:

1. Be Neat! Silver nitrate will stain skin, clothing, equipment, tables, walls, floors, and anything else it comes into contact with.
2. Always use highly purified water of at least 18 megaohm/cm resistance for all gel washings and incubations.
3. Avoid touching the gel with anything during the procedure. If the gel must be moved or touched, use a plastic disposable pipette or plastic forceps. Never touch the gel with bare hands. Powder free gloves should be worn at all times.
4. The gel can be washed, fixed, and stained in the same container. The container should be large enough for the gel to lie flat and to move slightly in any direction while in solution.
5. If the protocol has been altered to include acid in the gel fixation step, the acid must be removed completely before staining. A series of washes in 50% methanol alternating with deionized water effectively removes residual acid. The exact timing of these washes will depend on gel thickness, acrylamide concentration and how long it takes the gel to equilibrate in each solution. Be sure to wait until the gel has either shrunk (50% methanol) or swollen (water) maximally before changing. A general rule of thumb is that about 15-20 minutes are adequate for 1.0mm mini-gels, but additional time (up to one hour) may be necessary for thicker gels and will not be deleterious. Three or four cycles through methanol and water should be enough.
6. Maximum staining sensitivity is in the nanogram range. Stain development can be controlled by adding methanol to a final concentration of 5% - 10% in the Developing Solution. If the proteins show up rapidly and darken too quickly, the reaction rate can be controlled by adding methanol. The exact concentration of methanol is not critical; increases in methanol concentration correlate directly with decreases in reaction rate. After the most prominent bands appear and reach maximum intensity, the proteins present in lower concentrations begin to appear. During this time or slightly afterward, a general pale yellow background will also begin to appear. At this point, the reaction should be terminated by pouring off the Developing Solution and replacing it with Stop Solution.
7. **Important:** The Staining Solution contains silver and should not be left as an ammoniacal solution because of its explosive properties. The silver should be precipitated and the solution neutralized by the dropwise addition of 5% hydrochloric acid (HCl).[4] The liquid waste should be adjusted to a pH of 4-5 with sodium bicarbonate and disposed of in accordance with local regulations.
8. Gels are fragile and should not be subjected to physical stress while the various solutions are being removed from the gel container. Use a siphoning system for maximum security.
9. Stained gels can be stored for short periods of time (one or two days) before the stain quality starts to deteriorate. For more permanent storage, the gels can be dried onto cellophane dialysis membranes omitting exposure to heat. (Heat causes the stained protein bands to fade.) They can also be dehydrated by sequential exposure to higher concentrations of methanol (gradually increase the methanol to 100%) and stored in air-tight bags. The latter procedure is completely reversible if the concentration of water is gradually increased.
10. These procedures are directed specifically toward silver staining proteins. However, DNA and RNA also stain with greatly increased sensitivity over ethidium bromide staining.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

© Polysciences, Inc. 0904 RevB

Troubleshooting Guide:

1. High Background - High background is generally due to over-development of the stain or to residual chemicals in the gel. Over-development to compensate for too-low protein concentration causes non-specific background darkening. Unless the reaction is stopped using the methods just described, development may continue until the entire gel turns a dark brown color.

   Many chemicals used in PAGE affect the reaction and produce background staining. Urea, glycerol, ampholines, SDS, and non-specific reagent impurities all interfere with the reaction. They can reduce sensitivity and cause high background. Simply lengthening the fixation time can be very beneficial. These chemicals can be removed more thoroughly by washing the gel in 50% methanol alternating with water as described in the Gel Fixation sections. More wash cycles may be needed in some cases. If the gel contains Triton X-100, soak the gel for 2-3 hours with 3 changes of 50% methanol prior to the methanol-water wash cycle.

   If a high background does appear, it may be selectively removed by destaining the gel in Kodak Rapid Fix (film strength). This reversal process, if allowed to go to completion, will completely bleach the gel including the protein bands.

   To control the rate of the reaction, add methanol to the Rapid Fix so that objectionable background may be completely eliminated and the reaction terminated before there is noticeable loss of protein specific stain. Normally, 10% methanol in the Rapid Fix destaining solution is sufficient to provide the necessary control over the reaction. When Rapid Fix is used, it must be completely removed by a water wash, followed by a longer wash in a hypo-clearing agent such as Orbit Bath (TKO Chemical, St. Joseph, MO). Subsequently, the gel may be placed in 30% methanol. The bands or spots will be stabilized. The gel may be photographed and dried as usual. The intensity of the stained area is maintained with minimal fading when gels are dried without heat.

2. Surface Spots - Non-specific spots are caused by touching the gel with bare hands, by incomplete immersion of the gel in the various solutions, and by accidental exposure of the gel to contaminating chemicals.

   Protein deposits within the gel remain distinct, well-stained and unaffected by this gel surface phenomenon.

3. Silver Mirrors - Silver deposits (mirrors) may appear on the gel or on the container walls. Insufficient agitation during different stages of the staining procedure, adhering of the gel to the container, and impurities are the usual cause of gel mirrors.

4. Overload Samples - If too much protein has been loaded per sample, either shorten the development step or, if the gel has already been completely stained, bleach by using Kodak Rapid Fix, and restain while controlling the speed of reaction with methanol. The bleached gel can also be stained with Coomassie Blue.

5. Underloaded Samples - Despite the nanogram sensitivity of the silver staining procedure, some samples may still be in the minimally detectable range depending on the com position of the protein. Pre-staining with Coomassie Blue, followed by destaining the Coomassie Blue has been shown to increase sensitivity.

6. Other Unusual Non-specific Reactions - If any unusual reaction occurs at any point during the staining procedure, the gel can be recycled and reprocessed through the staining procedure. Use Rapid Fix to remove the silver and clear the gel, a hypo-clearing agent to remove the Rapid Fix, a water soak, and then soak in 50% methanol for gel re-equilibration before reprocessing. All these steps are essential for successful recycling of the gel for restaining.

   If the staining solution is not clear when it is prepared from Solutions A, B, and C, it may be titrated with a small amount of Solution B (NH_4OH) until it becomes clear.

Ordering Information:

Cat. #	Description	Size
16717-1	ElectroPure Silver Stain Kit	1kit

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484
In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
In Germany FAX: (49) 6221-764620

References:

1. Wray, W., et al., Anal. Biochem., 118, 197 (1981).
2. Lische, M.A., et al., Life Sciences, 25, 701 (1976).
3. Freeman, H.C., Inorganic Biochemistry, 1, 121 (1973).
4. “Procedure for Deactivation of Ammoniacal Silver Solution.” ORS Chemical Safety. Northwestern University: Office of the Vice President for Research, Sept. 2001.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

© Polysciences, Inc. 0904 RevB