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ProductInformation

SILVER STAIN KIT FOR POLYACRYLAMIDE GELS
Product Number **AG-25 AND AG-5**

TECHNICAL BULLETIN

Silver staining on polyacrylamide gels in both one- and two-dimensional gel electrophoresis systems is a highly sensitive visualization technique that allows detection of most proteins, DNA and RNA in the nanogram range. Generally, all silver staining techniques are 100-fold more sensitive than those using Coomassie Brilliant Blue R-250 (Coomassie Blue).[1] Therefore, silver staining is indispensable for detection of small amounts of proteins or poly-nucleotides, which cannot be seen with Coomassie Blue or without radioactive labels.

Current silver staining methods have had the disadvantages of long development time (12-24 hours), unstable solutions, and very toxic chemicals. Sigma's method is 3.5 hours from fixed to stained gel. Sigma's solutions are less toxic and stable as shipped. Sigma Silver Stain Kits (AG-5 and AG-25) are simple, reliable and comparable in sensitivity to other silver stain methods.[2] Sigma's Silver Stain Kit is based on the methodology of Hokeshoven and Dernick.[2] The method has given us reusable solutions and essentially colorless backgrounds in most gel electrophoresis systems. In addition to staining that is somewhat more quantitative than the commonly used method of Merril,[3] and the ability to stain over Coomassie Blue stained gels, Sigma's Silver Stain Kit is adaptable to both ultrathin layer (<0.5 mm) and conventional thicker slab polyacrylamide gels (up to 3.0 mm). The kits are also adaptable to nondenaturing gels, denaturing gels containing sodium dodecyl sulfate (SDS) and/or urea, and isoelectric focusing (IEF) gels. Sigma's method is not recommended for staining agarose or tube gels.

Sigma's Silver Stain Trial Pack (AG-5) is designed for staining 5 polyacrylamide gels to determine if this method is adaptable to your particular need(s). Sigma Silver Stain (AG-25) is designed for 25 polyacrylamide gels. Three gels may be stained per run in either kit with no adverse effects, thus tripling the number of gels that may be stained. However, some gels may require different development times for optimal visualization. Thus it may be advisable to develop each gel individually.

Each kit contains the following:
Plastic Development Tray
One Vial Silver Concentrate
One Vial Each of Developer 1 and 2
One Vial Each of Reducer A, B, and C

A laminated Procedure Card and a pamphlet with Procedures, Protocol and Helpful Hint Guide are included with the AG-25 and AG-5 kit.

Volumes of Reagent Concentrate Per Kit

Materials	Prod. No.	AG-5	AG-25
Silver Concentrate (Silver Nitrate)	S 3140	10 ml	50 ml
Developer 1 Concentrate (Sodium Carbonate)	D 4282	160 ml	750 ml
Developer 2 Concentrate (Formaldehyde)	D 4407	1.0 ml	5.0 ml
Reducer A Concentrate (Potassium Ferricyanide)	R 2006	15 ml	75 ml
Reducer B Concentrate (Sodium Thiosulfate)	R 2131	25 ml	110 ml
Reducer C Concentrate (Sodium Carbonate)	R 2256	5 ml	25 ml
Plastic Development Tray	P 2915	1 ea	1 ea

Other Reagents Needed:
Ethanol (Product No. 24,511-9)
Acetic Acid (Product No. 10,908-B)
Glycerol [for drying gels (G 9012)]

Trichloroacetic Acid (T 4885) [For IEF gel staining]
In addition, we recommend 25% Glutaraldehyde Solution (G 6257) and sodium acetate (S 9513) for ultrathin IEF polyacrylamide gels.

Sigma Silver Stain is approximately 100 times more sensitive than Coomassie Blue and up to 2 times more sensitive than Ethidium Bromide. Protein levels should be adjusted accordingly to prevent overloading problems. We have found 10 to 50 nanograms of most proteins to be detectable on nondenaturing and denaturing gels, and 120-300 nanograms to be detectable on IEF gels, but this may vary with your particular protein.

Silver Stain Reagents
NOTE: For minigels, the volumes below can be reduced by one-half.

Fixing Solution
A. Non-IEF Gels
A 30% ethanol (EtOH)-10% glacial acetic acid (HAc) solution is prepared for the fixing process by adding 90 ml of HAc to 270 ml of EtOH and adjusting to a final volume of 900 ml with deionized water (dH_2O).

B. IEF Gels
A 20% Trichloroacetic Acid (TCA) solution is prepared by adding 180 grams of TCA to 500 ml of dH_2O and adjusting to a final volume of 900 ml with dH_2O.

Silver Equilibration Solution
A. Gels of 0.5 mm thickness or greater (conventional)—dilute 1.5 ml of Silver Concentrate to 300 ml with dH_2O.

B. Gels less than 0.5 mm thickness (ultrathin)—dilute 0.75 ml of Silver Concentrate to 300 ml with dH_2O. (This solution may be used twice before discarding or salvaging the silver.)

Development Solution
Dilute 30 ml of Developer 1 Concentrate to 300 ml with dH_2O. To this solution, add 0.17 ml of Developer 2 Concentrate. (This solution is stable for up to 2 hours.)

NOTE: If Developer 1 Concentrate contains precipitate or has crystallized, see Helpful Hint Section.

Stop Solution
Make a 1% Acetic Acid solution by adding 3 ml of Glacial Acetic Acid to 297 ml dH_2O.

Reducer Solution
Mix together 2.0 ml of Reducer A Concentrate, 4.0 ml of Reducer B Concentrate and 0.7 ml of Reducer C Concentrate and then dilute to 300 ml with dH_2O.

(This solution will be stable for 1 day or until the solution turns green or blue.)

Procedure For Silver Staining Polyacrylamide Gels

To stain over Coomassie stained gels or Ethidium Bromide stained gels begin with Step 2

Volumes may be reduced by one-half for staining minigels.

All steps are to be performed at 21-25 °C and require constant gentle agitation.

Always wear clean gloves; fingerprints on gels are detectable.

1. Fixing
A. Non-IEF Gels
After removal from the electrophoresis chamber, the gels should promptly be placed in the EtOH-HAc Fixing Solution.

For conventional gels, fix three times with 300 ml each changing every 20 minutes (60 minutes total). For ultrathin gels, the changes are made every 10 minutes (30 minutes total).

Pour off solutions and proceed with Step 2.

Note: Gels may also be fixed in 12.5% (TCA)—3.5% Sulfosalicylic acid or the traditional methanol method (40% Methanol—7% HAc and 53% water) but must then be thoroughly equilibrated in dH_2O in Step 2.

B. IEF Gels
After removal from the electrophoresis chamber, the gels should promptly be placed in the TCA Fixing Solution.

For conventional gels, fix three times with 300 ml each changing every 20 minutes (60 minutes total).

For ultrathin gels, fix with three 300 ml changes every 10 minutes (30 minutes total) followed immediately by 300 ml of 2.5% Glutaraldehyde in 1 M Sodium Acetate for 30 minutes.

Pour off solutions and proceed with Step 2. The Note from 1A applies here also.

2. Water Rinse
For conventional gels, rinse three times with 300 ml of deionized water for 10 minutes each rinse (30 minutes total). For ultrathin gels, rinse three times with 300 ml of deionized water for 5 minutes each rinse (15 minutes total). Pour off water when finished.

3. Silver Equilibration
Place conventional or ultrathin gels in 300 ml of appropriate Silver Equilibration Solution and allow it to equilibrate with gentle agitation for 30 minutes. Pour off the solution before proceeding to Step 4.

4. Rapid Water Rinse
The conventional and ultrathin gels are rinsed for 10-20 seconds with 300 ml of dH_2O. Pour water off when finished.

5. Development of Gel
Place one-half of the 300 ml of Developer Solution over the gel. After 5-8 minutes discard the first half of the Developer Solution and add the remaining Developer Solution. Watch carefully to achieve the darkest bands while minimizing the yellowing or darkening of the gel.

6. Stopping The Development
Pour off the Developer Solution and rapidly replace with Stop Solution for 5 minutes.

7. Water Rinse
For conventional gels, rinse three times with 300 ml of dH_2O for 10 minutes each rinse (30 minutes total). For ultrathin gels, rinse three times with 300 ml of dH_2O for 5 minutes each rinse.

8-9. Reducer Wash / Tap Water Rinse
Place gel(s) in 300 ml of the Reducer Solution for 10-30 seconds. Remove Reducer Solution from gel and immediately rinse under running tap water for 1 minute.

10. Water Rinse
For conventional gels, rinse three times with 300 ml of dH_2O for 10 minutes each rinse. For ultrathin gels, rinse three times with 300 ml of dH_2O for 5 minutes each rinse.

11. Recycle Option
If increased sensitivity is desired, recycle the gel through the staining procedure starting with Step 3 and proceed through Step 10.

12. Storage
Store in dH_2O indefinitely or dry after equilibration in 2% aqueous Glycerol Solution. Gel band(s) have not faded in our studies for up to three months.

SILVER STAIN PROTOCOL

			Duration	
			Ultrathin	**Conventional**
	Reagent	Volume	0.5 mm	0.5-3.0 mm
1.	Fixing	300 ml x 3	10 min. x 3	20 min. x 3
2.	Rinse with dH2O	300 ml x 3	5 min. x 3	10 min. x 3
3.	Silver Equilibration Solution	300 ml	30 min.	30 min.
4.	dH2O Rinse	300 ml	10-20 sec.	10-20sec.
5.	Development Solution	150 ml x 2	5-8 min. x 2	5-8 min.x2
6.	Stop Solution	300 ml	5 min.	5min.
7.	dH2O Rinse	300 ml x 3	5 min. x 3	10 minx 3
8.	Reducer Solution`	300 ml	10-30 sec.	10-30 sec
9.	Rinse with tap water	Running water	1 min.	1 min.
10.	dH2O Rinse	300 ml x 3	5 min. x 3	10minx3
11.	Recycle (option)	Repeat from Step 3 through Step 10.		
12.	Storage	Store in dH_2O indefinitely or dry the gel after an equilibration of 2 hours in 2% aqueous Glycerol.		

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Bands do not develop.	Temperature of solution too low.	If temperature of solution is not 21-25 °C, the development reaction will not work properly.
	Gel not washed thoroughly enough	Possibly not all of the chemicals that could interfere with the silver staining development were completely removed.
	Plates were not clean.	Clean plates with a detergent wash or nitric acid : water (1:1) overnight.
	Didn't fix properly	Check Fixing Solution or fixing properties of your particular protein(s).
Band sensitivity too low or loss of sensitivity over time.	Solution temperature too low.	Reactions must occur at 21-25 °C.
	Too long of exposure to Reducer Solution	The Reducer Solution, if not thoroughly remove will bleach the gel totally if allowed to exceed 20 seconds. Bleaching totally may result in some decrease in sensitivity in the bands.
	Under-development.	Recycle as described in the Stain Protocol.
	Excessive water wash after Silver equilibration.	Wash only in 300 ml of deionized water for 10-15 sec.
	Bands too diffuse.	Too low protein, must have 10-100 nanograms per band depending upon properties of some proteins.**
	Plates not clean.	Wash with detergent or nitric acid and water (1:1) overnight.
Background is excessively dark (over-developed).	Too long of exposure to Developer Solution	Usually no more than 10-15 minutes is required for development of bands at 21-25 °C.*
	Washing not complete.	Try three 300 ml of 10% ethanol wash for 10 minutes each rinse. This step can be inserted between the fixing step and the deionized water rinse.
	Water impure or contaminated.	It is best to use deionized water when possible.

TROUBLESHOOTING GUIDE (continued)

Problem	Cause	Solution
	Impure acrylamide.	Use Sigma 99+% Acrylamide (A 9099) and 98+% bis-Acrylamide (M 2022).
Bands lighter than the background.	Overloading.	Lower amount of protein loaded onto the gel.
Splotchy background or hazy mirroring.	Reducer Solution not used or not used properly.	If the Reducer Solution isn't used, some haze may develop. Check to make sure the Reducer Solution was diluted and thoroughly removed after exposure.
	Gel surface not clean.	Wear gloves at all times while handling gels because fingerprints will show in gels.
Developer Solution has precipitated.	Cold may cause precipitation	Heat to 50 °C until dissolved.
Smearing or blackening in lanes where protein loaded	Overloading.	Remember Silver Staining blackening is approx. 50-100 more sensitive than Coomassie Blue (Dilute samples accordingly

* If high background staining occurs, it may be selectively removed and the intensity of the stained deposits may be lightened or removed by longer exposure of the gels to the Reducer Solution. This reversal process, if allowed to go to completion, will completely bleach the gel. Restaining after bleaching with Reducer Solution is possible with little or no sensitivity decrease by beginning with step 2 of the procedure, after a thorough water wash.

** Some increased sensitivity has been achieved by prestaining with Coomassie Blue followed by the routine destaining and beginning with step 2 of the Silver Stain Procedure. This increases sensitivity two- to five-fold and helps increase the detectability of some minimally detectable proteins.

References:

1. Ochs, D.C., McConkey, E.H., and Sammons, D.W., Electrophoresis, **2**, 304 (1981).
2. Heukeshoven, J., and Dernick, R., Electrophoresis, **6**, 103 (1985).
3. Merril, C.R., Goldman, D., Sedman, S.A., and Ebert, M.H., Science, **211**, 1437 (1981).

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