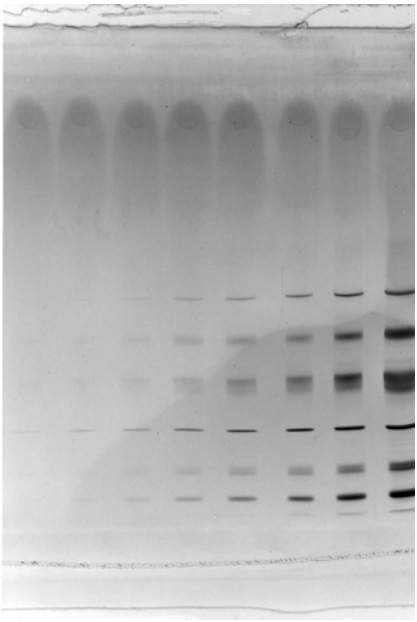
instructions

Silver Staining Kit Protein



ⓘ 71-7177-00 Edition AF

Amersham Biosciences

1. Introduction

Silver Staining Kit, Protein is a reagent kit for the fast, easy, reproducible and sensitive staining of proteins in polyacrylamide gels. Ready-to-use solutions and pre-weighed chemicals minimise preparation time and maximize ease of use and reliability. Proteins can be visualized in about 2 hours.

The kit, which is part of the PlusOne range of electro-phoresis chemicals, is based on the methodology of Heukeshoven and Dernick (1).

The high sensitivity of the visualization technique allows detection of most proteins down to the nanogram range, which is 100 times more sensitive than Coomassie Brilliant Blue. The method is reliable and reproducible and gives essentially colourless backgrounds in most gel electrophoresis systems.

Silver Staining Kit, Protein is developed for use with Amersham Biosciences precast gels as well as with thicker, conventional mini slab polyacrylamide gels (thickness up to at least 1.5 mm). The kit is thus suitable for non-denaturing gels, denaturing gels containing sodium dodecyl sulphate (SDS) and/or urea, and isoelectric focusing (IEF) gels.

1 Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. Electrophoresis 6 (1985) 103–112, Heukeshoven, J. and Dernick, R.

2. Kit contents and technical data

Kit contents

Silver Staining Kit, Protein (Code No. 17-1150-01) contains the following items. Quantities are sufficient to stain 10 precast gels (12.5 x 26 cm) or 20 mini slab gels (8–10 x 8 cm).

Item	Quantity
Sodium acetate	10 x 17 g packets
5% Sodium thiosulphate	1 x 100 ml bottle
EDTA-Na2	10 x 3.65 g packets
Sodium carbonate	10 x 6.25 g packets
2.5% Silver nitrate	2 x 125 ml bottles
37% Formaldehyde	1 x 2.5 ml bottle
25% Glutardialdehyde	1 x 12.5 ml bottle

Technical data

Sensitivity:	0.2–0.6 ng protein per band
Storage:	+10 to +30 °C
Shelf life:	1 year at recommended storage conditions

Precautions: The chemicals in this kit should not be discarded via public waste water systems. Please dispose of these chemicals properly. Consult your local regulations for more information.

Read the warning text on the label of each bottle and packet.

3. Protocol and procedure for silver staining

General information

This protocol is optimized for the following precast gels from Amersham Biosciences: ExcelGel SDS Homogeneous 7.5, 12.5 and 15, ExcelGel SDS, gradient 8–18, ExcelGel XL SDS 12–14, CleanGel, Immobiline DryPlate, as well as for mini slab gels.

Wear gloves at all times. Perform all steps at 21 to 25 °C with constant gentle agitation on a shaker. We recommend using Staining Tray 1 or Staining Tray 2 (see Accessories, p. 9). These two accessories have polished surfaces of SIS 2332 grade stainless steel that do not interfere with the silver staining technique.

Each packet of chemicals in this kit is made up to a 250 ml solution. 250 ml of solution is needed per pre-cast gel (12.5 × 26 cm) except ExcelGel XL SDS 12–14, which requires 400 ml. Mini slab gels (8–10 × 10 cm) require 125 ml of solution.

The quality of the water used for making up the solutions and for washing the gel will affect the staining result. For best results use water with a conductivity of 5 megaOhms or less.

A. Protocol for Pharmacia Biotech precast gels (ExcelGel SDS, CleanGel and Immobiline DryPlate) and mini slab gels

Chemicals required

- Chemicals included in this Silver Staining Kit, Protein, plus:
- Ethanol (=95%)
- Glacial acetic acid
- Glycerol (87% w/w) (1000 ml) (Code No. 17-1325-01)

Reagents

Freshly made solutions (not older than 24 hrs) give best results. Make up the following reagent solutions using the chemicals listed above (add components marked * immediately before use):

Fixing solution:	Ethanol	100 ml
	Glacial acetic acid	25 ml
	Make up to 250 ml with distilled water.	
Sensitizing solution:	Ethanol	75 ml
	Glutardialdehyde (25% w/v)*	1.25 ml
	Sodium thiosulphate (5% w/v)	10 ml
	Sodium acetate (17 g)	1 packet
	Make up to 250 ml with distilled water.	
Silver solution:	Silver nitrate solution (2.5% w/v)	25 ml
	Formaldehyde (37% w/v)*	0.1 ml
	Make up to 250 ml with distilled water.	
Developing solution:	Sodium carbonate (6.25 g)	1 packet
	Formaldehyde (37% w/v)*	0.05 ml
	Make up to 250 ml with distilled water.	
	Stir vigorously to dissolve the sodium carbonate.	
Stop solution:	EDTA-Na2•2H2O (3.65 g)	1 packet
	Make up to 250 ml with distilled water	
Washing solution:	Distilled water	
Preserving solution for plastic backed gels:	Glycerol (87% w/w)	25 ml
	Make up to 250 ml with distilled water	
Preserving solution for gels not supported on plastic films:	Ethanol	75 ml
	Glycerol (87% w/w)	11.5 ml
	Make up to 250 ml with distilled water	

B. Protocol for Ampholine PAG plate

This protocol is optimized for Ampholine PAGplate precast gels.

Chemicals required

- Chemicals included in this Silver Staining Kit, Protein, plus:
- Ethanol (=95%)
- Trichloroacetic acid (TCA)
- Glycerol (87% w/w) (1000 ml) (Code No. 17-1325-01)

Reagents

Freshly made solutions (not older than 24 hrs) give best results. Make up the following reagent solutions using the chemicals listed above (add components marked * immediately before use):

Fixing solution:	Trichloroacetic acid (TCA)	50 g
	Dissolve and make up to 250 ml with distilled water.	
Sensitizing solution:	Ethanol	75 ml
	Glutardialdehyde (25% w/v)*	1.25 ml
	Sodium thiosulphate (5% w/v)	10 ml
	Sodium acetate (17 g)	1 packet
	Make up to 250 ml with distilled water.	
Silver solution:	Silver nitrate solution (2.5% w/v)	25 ml
	Formaldehyde (37% w/v)*	0.1 ml
	Make up to 250 ml with distilled water.	
Developing solution:	Sodium carbonate (6.25 g)	1 packet
	Formaldehyde (37% w/v)*	0.05 ml
	Make up to 250 ml with distilled water.	
	Stir vigorously to dissolve the sodium carbonate.	
Stop solution:	EDTA-Na2•2H2O (3.65 g)	1 packet
	Make up to 250 ml with distilled water.	
Washing solution:	Distilled water	
Preserving solution:	Glycerol (87% w/w)	25 ml
	Make up to 250 ml with distilled water.	

Staining procedure

250 ml of solutions are needed per 12.5 × 26 cm precast gel and 125 ml per 8–10 × 8 cm mini slab gel. The staining time is 2 hours. All steps should be performed with gentle shaking of the staining tray.

Fixation: 30 min.

Soak the gel in fixing solution for 30 minutes.

Note: The next step is for Ampholine PAGplate only. For other gels, proceed to "Sensitizing".

Washing: 3 x 5 min.

Wash the Ampholine PAGplate gel three times in distilled water for 5 minutes each time.

Sensitizing: 30 min.
Remove the solution. Add sensitizing solution and leave shaking for at least 30 minutes.

Washing: 3 x 5 min.
Remove the sensitizing solution. Add distilled water and wash three times for 5 minutes each time. (Note: Wash three times for 10 minutes each time for Ampholine PAGplate gels.)

Silver reaction: 20 min.
Add silver solution and leave shaking for 20 minutes.

Washing: 2 x 1 min.
Remove the silver solution. Rinse twice in distilled water for one minute each time.

Developing: 2–5 min.
Add developing solution and leave shaking for 2–5 minutes.

Stopping: 10 min.
Remove the developing solution. Add stop solution and leave shaking for 10 minutes.

Washing: 3 x 5 min.
Remove the stop solution. Add distilled water and wash three times for 5 minutes each time.

Preserving: 20 min. (plastic-backed gels) 2 x 30 min. (gels not supported on plastic films)
Add preserving solution and leave shaking for 20 minutes for plastic backed gels. For gels not supported on plastic films, shake for 30 minutes, pour off the solution, add fresh and shake for a further 30 minutes.

Drying: Overnight
Put the gel on a glass plate (treat the plate first with RepelSilane for gels not supported on plastic films) and wrap it in Cellophane Sheet. Leave the gel to dry overnight at room temperature. Do not put the gel in a heating cabinet (the silver stain bleaches at elevated temperatures).

4. Trouble shooting guide

Problem	Cause	Remedy
bands do not develop	temperature of solution too low	Keep temperature of solution between 5 and 25 °C
	Gel not washed thoroughly enough	Repeat wasing procedure
	Plates were not clean	Clean plateswith a detergent wash or nitric acid:water (1:1) overnight
	Did not fix properly	Check fixing solution or fixing properties of your particular protein(s)
Background is excessively dark (over-developed)	Temperature of developing solution too high	Chill the developing solution in the refrigerator before use
	Water impure or contaminated	Use deionized water whenever possible
	Ampholine PAGplate carrier ampholytes have not been washed out	After stop solution step, put the gel in 1% sodium thiosulphate solution for several hours to reduce the silver stain
Bands lighter than the background	Overloading	Lower the amount of protein loaded onto the gel
Smearing or blackening in lanes where protein loaded	Overloading	Dilute samples (remember silver staining is approx. 50–100 times more sensitive than Coomassie Blue)
Silver mirror reaction	Insufficient washing with water	Extend the time of washing after staining
	Dirty tray	Replace the chamber for development with a new clean one
	Insufficient shaking	During the development step shake the tray sufficiently to prevent the gel from sticking to the bottom of the tray

5. Other chemicals and accessories

Chemicals	Code No.
Ethanol	–
Glacial acetic acid	–
Trichloroacetic acid (TCA)	–
Glycerol (87% w/v) (1000 ml)	17-1325-01
RepelSilane (500 ml)	17-1331-01

Accessories	Code No.
Staining Tray 1 with lid and removable gel holder (60 x 150 x 300 mm)	18-1018-08
Staining Tray 2 with lid (60 x 260 x 320 mm)	18-1018-09
Cellophane Sheets	80-1129-38

6. Precast gels and related products

Molecular weight and pI markers

	Code No.
MW range 2,512–16,949	80-1129-83
MW range 14,000–94,000	17-0446-01
MW range 53,000–212,000	17-0615-01
MW range 67,000–670,000	17-0445-01
Broad pI kit pH 3.5–9.3	17-0471-01
Low pI kit pH 2.8–6.5	17-0472-01
High pI kit pH 5.2–10.3	17-0473-01
Carbamalyte calibration kit	17-0582-01

Precast gels and buffer strips

SDS PAGE and Native PAGE

	Code No.
ExcelGel SDS Homogeneous 7.5	80-1260-01
ExcelGel SDS Homogeneous 12.5	80-1261-01
ExcelGel SDS Homogeneous 15	80-1262-01
ExcelGel SDS, gradient 8–18	80-1255-53
ExcelGel XL SDS 12–14	17-1236-01
ExcelGel SDS Buffer Strips	17-1342-01
CleanGel 25S	18-1031-54
CleanGel 36S	18-1031-55
CleanGel 48S	18-1031-56

IEF

	Code No.
CleanGel IEF	18-1035-32
Ampholine PAGplate pH 3.5-9.5	80-1124-80
Ampholine PAGplate pH 4.0-6.5	80-1124-81
Ampholine PAGplate pH 5.5-8.5	80-1124-82
Ampholine PAGplate pH 4.0-5.0	80-1124-83
Immobiline DryPlate pH 4-7	80-1128-28
Immobiline DryPlate pH 4.2-4.9	80-1128-29
Immobiline DryPlate pH 4.5-5.4	80-1128-30
Immobiline DryPlate pH 5.0-6.0	80-1128-31
Immobiline DryPlate pH 5.6-6.6	80-1128-32

2-D

First dimension

	Code No.
110 mm	
Immobiline DryStrip pH 4–7 L	18-1016-60
Immobiline DryStrip pH 3–10 L	18-1016-61
180 mm	
Immobiline DryStrip pH 4–7 L	17-1233-01
Immobiline DryStrip pH 3–10 L	17-1234-01
Immobiline DryStrip pH 3–10 NL	17-1235-01
Second Dimension	
ExcelGel SDS, gradient 8–18	80-1255-53
ExcelGel XL SDS 12–14	17-1236-01
ExcelGel SDS Buffer Strips	17-1342-01

Important Information

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