

PageSilver™ Silver Staining Kit

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COMPONENTS OF THE KIT

PageSilver™ Silver Staining Kit	#K0681 for 25 mini-gels
Sensitizer concentrate	15 ml
Staining reagent	100 ml
Developing reagent	250 ml
Formaldehyde	2.1 ml
Slop reagent	200 ml

STORAGE

All components of the kit should be stored at room temperature. The kit is stable for 12 months at room temperature.

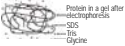





DESCRIPTION

The PageSilver™ Silver Staining Kit is a complete system for rapid and sensitive staining of proteins, DNA and RNA in polyacrylamide gels. The staining procedure is fast and simple and can be completed in approximately 60 minutes with very little hands-on time. The composition of all components is optimized for highly sensitive protein staining. Approximately, 0.05-0.6 ng of protein per band can be visualized in an essentially clear background. This is >100 times more sensitive than traditional Coomassie Brilliant Blue R250 staining and allows for superior detection of low abundance proteins. The kit enables visualization of nanogram quantities of DNA and RNA.

The PageSilver™ Silver Staining Kit is based on the use of silver nitrate to bind proteins at a weakly acidic pH and subsequent reduction of silver ions to metallic silver by formaldehyde at an alkaline pH (1, 2).

The kit is suitable for staining both non-denaturing polyacrylamide gels and denaturing polyacrylamide gels containing SDS and/or urea. Subsequent analysis of stained protein bands by mass spectrometry is not recommended.

OVERVIEW OF STAINING PROCEDURE

Staining step	Description	Schematic view
Gel fixing 1	Ethanol and acetic acid in the Gel fixing solution 1 fix the proteins to the gel matrix to avoid protein diffusion. All the interfering ions from electrophoresis buffer (SDS, Tris, Glycine) are removed.	
Gel fixing 2	Gel fixing solution 2 removes acetic acid used in Gel Fixing 1 step, proteins remain fixed in the gel matrix.	
Sensitizing	Components of the Sensitizing solution interact with proteins and the gel to increase the sensitivity and contrast of staining. Washing with ultrapure water removes excess of sensitizer.	
Staining	Silver ions present in the Staining solution saturate the gel and protein molecules. Washing with ultrapure water removes silver ions from the gel matrix. Silver ions remain on proteins due to interactions with the negatively charged amino acid residues.	
Developing	Developing solution reduces protein-bound silver ions to metallic silver and leads to the development and visualization of protein bands.	
Terminating	Stop solution terminates silver ion reduction to avoid high background due to prolonged developing. At this point gel can be scanned or dried for long term storage.	

ADDITIONAL MATERIALS REQUIRED

- Deionized water (>18 MΩ/cm recommended)
- Glass or plastic tray, microwavable
- Teflon-coated stir bars
- Clean glass bottles for reagent preparation
- Graduated glass cylinder
- Rotary shaker
- Microwave oven (700-1200 W)
- Ethanol 96-100%
- Glacial acetic acid

IMPORTANT NOTES

- To prevent silver deposits on staining trays and inconsistent staining, all containers used for mixing and staining should be clean. After cleaning with laboratory detergent, we recommend additional cleaning of glass or polypropylene containers with 50% nitric acid. Rinse thoroughly with high quality deionized water.
- Wear gloves that have been rinsed with deionized water while handling gels. Never touch gels with metal objects or bare skin to avoid keratin contamination. Perform gel manipulations with glass rods where possible.
- Use containers that allow full immersion and free movement of the gel during agitation.
- Ensure that the gel is completely submerged in solution. Gel floatation on the surface of the solution may lead to inconsistent staining and background discoloration.
- Avoid staining gels in direct sunlight or at temperatures above 25°C.
- Adjust the temperature of the deionized water to 20-25°C before use.
- If the acrylamide contains dust particles, this will lead to artifacts after silver staining. To obtain high quality electrophoresis and silver staining results, the acrylamide solution that is used for gel preparation should be filtered through a membrane with 0.2 μm pore size.
- Use freshly prepared solutions.
- Do not exceed 50 mM DTT in the protein sample prepared for loading. If initial protein sample contains >50 mM DTT, do not add DTT into sample loading buffer (e.g. do not add Reducing Agent when using #R0891 or #R1011).
- Coomassie Brilliant Blue (CBB) stained gels can be additionally silver stained to achieve higher sensitivity. No destaining of CBB is needed, proceed directly with the silver staining protocol. Destaining occurs during the first two silver staining protocol steps.
- Fermentas Unstained and Prestained PageRuler™ Protein Ladders (#SM0661, #SM0671 and #SM1811) can be successfully stained with PageSilver™ Silver Staining Kit. To avoid overloading, all protein ladders should be used in considerably lower amounts compared to Coomassie Blue stained gels. Prepare ladder loading mix immediately prior to use:

Water, nuclease-free (#R0581)	36.5 μl
5X Loading Dye (#R0891)	10 μl
20X Reducing Agent (#R0891)	2.5 μl
Protein Ladder	1 μl

 Load 5 μl of the mixture on SDS polyacrylamide gel.

PREPARATION OF WORKING SOLUTIONS

1. Preparation of fixative solutions

Prepare the following two gel fixing solutions as outlined. The provided volumes are sufficient for staining of one mini-gel (8 x 10 cm, 0.75-1.5 mm thick). The prepared solutions can be stored in tightly closed bottles at room temperature.

Add the reagents to the clean glass bottles.

Reagent	Gel fixing solution 1		Gel fixing solution 2		
	Volume	Final concentration	Volume 1	Volume 2	Final concentration
Ethanol	50 ml	50% (v/v)	60 ml	90 ml	30% (v/v)
Glacial acetic acid	10 ml	10% (v/v)	-	-	-
Water, ultrapure	40 ml	-	140 ml	210 ml	-
Total volume	100 ml	-	200 ml	300 ml	-

Volume 1 – for Staining Protocol for Maximum Speed (p.6-7).

Volume 2 – for Staining Protocol for Maximum Sensitivity (p.8-9).

2. Preparation of staining solutions

Prepare the following four gel staining solutions using reagents provided in the kit. For best results the solutions should be prepared the same day prior to staining. Add the formaldehyde only immediately prior to staining.

The provided volumes are sufficient for staining one mini-gel (8 x 10 cm, 0.75-1.5 mm thick).

Mark clean glass bottles and add the indicated volumes of reagents.

Reagent	Sensitizing solution	Staining solution	Developing solution	Stop solution
Sensitizer concentrate	0.4 ml	-	10 µl	-
Staining reagent	-	4 ml	-	-
Developing reagent	-	-	10 ml	-
Stop reagent	-	-	-	8 ml
Water, ultrapure	to 100 ml	to 100 ml	to 100 ml	92 ml
Formaldehyde	-	54 µl*	27 µl*	-
Total volume	100 ml	100 ml	100 ml	100 ml

* Add immediately prior to use.

Note. Check the Developing reagent for salt precipitation before use. Redissolve precipitate by warming the solution at 37°C then cool back to 25°C before use.

STAINING PROTOCOL FOR MAXIMUM SPEED

Protocol for maximum speed of protein staining, enables visualization of 0.1 ng of protein per band in approximately an hour. Involves microwave steps.

Step	Procedure
1	<p>Gel fixing 1</p> <ul style="list-style-type: none"> Place the gel into a staining tray and rinse briefly with deionized water. Add 100 ml of Gel fixing solution 1 and microwave uncovered for 30 s at high power. Do not boil! Remove the tray from the microwave oven and gently agitate for 10 min. <p>Note: staining can be halted at this step. Leave the gel in the Gel fixing solution 1 overnight if there is not enough time to complete the staining protocol.</p> <ul style="list-style-type: none"> Discard the Gel fixing solution 1.
2	<p>Gel fixing 2 and Washing</p> <p>Perform Gel fixing 2 procedure twice:</p> <ul style="list-style-type: none"> Add 100 ml Gel fixing solution 2 and microwave uncovered for 30 s at high power. Do not boil! Remove the tray from the microwave oven and gently agitate for 10 min. Discard the Gel fixing solution 2. <p>Perform Washing procedure twice:</p> <ul style="list-style-type: none"> Add 100 ml of deionized water and gently agitate for 20 s. Discard the water.
3	<p>Sensitizing and Washing</p> <ul style="list-style-type: none"> Add 100 ml of Sensitizing solution and gently agitate for 1 min. Discard the Sensitizing solution. <p>Perform Washing procedure twice:</p> <ul style="list-style-type: none"> Add 100 ml of deionized water and gently agitate for 20 s. Discard the water.

Step	Procedure
4	<p>Staining and Washing</p> <ul style="list-style-type: none"> Add 100 ml of Staining solution and gently agitate for 20 min. Discard the Staining solution. <p>Perform Washing procedure twice:</p> <ul style="list-style-type: none"> Add 100 ml of deionized water and gently agitate for 20 s. Discard the water. <p>Note: do not exceed one minute to avoid removing silver ions from the gel and decreasing the staining sensitivity.</p>
5	<p>Developing</p> <ul style="list-style-type: none"> Add 100 ml of Developing solution and gently agitate for approximately 4 min until bands are well-developed and the required intensity is reached. Discard the Developing solution. <p>Note: for staining DNA and RNA bands, develop for 12-20 min.</p>
6	<p>Terminating</p> <ul style="list-style-type: none"> Add 100 ml of Stop solution and gently agitate for 5 min. Discard the Stop solution. <p>Note: store the gel in deionized water. If required, dry the gel according to recommendations on p. 9.</p>

STAINING PROTOCOL FOR MAXIMUM SENSITIVITY

Protocol for maximum sensitivity of protein staining, enables visualization of 0.05 ng of protein per band in approximately 2 h 40 min at room temperature. Does not involve microwave steps.

Step	Procedure
1	<p>Gel fixing 1</p> <ul style="list-style-type: none"> Place the gel into a staining tray and rinse briefly with deionized water. Add 100 ml of Gel fixing solution 1 and gently agitate for 60 min. <p>Note: staining can be halted at this step. Leave the gel overnight in the Gel fixing solution 1 if there is not enough time to complete the staining protocol.</p> <ul style="list-style-type: none"> Discard the Gel fixing solution 1.
2	<p>Gel fixing 2 and Washing</p> <p>Perform Gel fixing 2 procedure three times:</p> <ul style="list-style-type: none"> Add 100 ml Gel fixing solution 2 and gently agitate for 20 min. Discard the Gel fixing solution 2. <p>Perform Washing procedure twice:</p> <ul style="list-style-type: none"> Add 100ml of deionized water and gently agitate for 20 s. Discard the water.
3	<p>Sensitizing and Washing</p> <ul style="list-style-type: none"> Add 100 ml of Sensitizing solution and gently agitate for 1 min. Discard the Sensitizing solution. <p>Perform Washing procedure twice.</p> <ul style="list-style-type: none"> Add 100 ml of deionized water and gently agitate for 20 s. Discard the water.

Step	Procedure
4	Staining and Washing <ul style="list-style-type: none"> Add 100 ml of Staining solution and gently agitate for 20 min. Discard the Staining solution.
	Perform Washing procedure twice : <ul style="list-style-type: none"> Add 100 ml of deionized water and gently agitate for 20 s. Discard the water. <i>Note: do not exceed one minute to avoid removing silver ions from the gel and decreasing the staining sensitivity.</i>
5	Developing <ul style="list-style-type: none"> Add 100 ml of Developing solution and gently agitate for 5-10 min until bands are well-developed and the required intensity is reached. Discard the Developing solution. <i>Note: for staining DNA and RNA bands, develop for 12-20 min.</i>
	Terminating <ul style="list-style-type: none"> Add 100 ml of Stop solution and gently agitate for 10 min. Discard the Stop solution. <i>Note: store the gel in deionized water. If required, dry the gel according to recommendations below.</i>
6	

GEL DRYING

Add 100 ml of gel drying solution (10 ml glycerol, 15 ml ethanol, 75 ml water) and agitate for 30 min. Dry wrapped in cellophane film.
Alternatively the gel can be dried on a filter paper with a gel-dryer.

TROUBLESHOOTING

Problem	Cause and Solution
Bands develop poorly or not at all	Staining solutions prepared incorrectly Remake all solutions according to directions on p.5. Ambient temperature is below 20°C Prolong development time by 25%. Temperature of the solutions is too low Make sure the working solution temperatures are of room temperature (20-25°C). Plates were not clean Clean plates with detergent or soak in nitric acid:water solution (1:1) overnight. The gel is too thin (<0.75 mm) Shorten Washing step after staining to avoid removing of silver ions prior development. Loss of silver ions from the gel Do not exceed wash for more than 60 seconds after staining. Low protein load Increase the amount of total protein loaded on the gel to at least 1-5 ng.
	Negative staining (larger band with a pale area in the middle) Protein band is overloaded Decrease protein amount per band. Do not overload protein ladder lane (see p. 4)
Dark or uneven background	Poor water quality Use deionized water of >18 MΩ/cm resistance. Staining trays insufficiently cleaned Use staining trays dedicated for silver staining. After silver staining, wash trays with detergent and water, and rinse them with deionized water. Improper washing. Follow directions on number and duration of washes. Gels are not completely submerged during staining Be sure to completely immerse gels in staining solution and perform all steps using a rotary shaker to ensure even staining.

Problem	Solution
Streaking or yellow background near the top of the gel	High concentration of DTT in the sample Use 30-50 mM DTT for reducing your samples. If initial protein sample contains >50mM DTT, add sample loading buffer without DTT (e.g. #R0891)
50-70 kDa band present in all lanes of the gel	Keratin contamination from skin Wear gloves when preparing electrophoresis buffers, handling electrophoresis chambers, combs and gels. Rinse wells of the gel with ultrapure water before loading.

QUALITY CONTROL

The PageSilver™ Silver Staining Kit is tested using the Staining Protocol for maximum speed, different dilutions of BSA (0.1 - 2.0 ng) are electrophoresed on a 1.0 mm, 12% Tris-glycine SDS gel. Silver staining detects 0.1 ng BSA in clear background.

RELATED PRODUCTS

Product	Amount	Catalog #
Spectra™ Multicolor Broad Range Protein Ladder	2 x 250 µl	SM1841
PageRuler™ Unstained Protein Ladder	2 x 250 µl	SM0661
PageRuler™ Prestained Protein Ladder	2 x 250 µl	SM0671
PageRuler™ Prestained Protein Ladder Plus	2 x 250 µl	SM1811
Unstained Protein Molecular Weight Marker	2 x 1000 µl	SM0431
Prestained Protein Molecular Weight Marker	2 x 250 µl	SM0441
PageBlue™ Protein Staining Solution	1 liter	R0571
DualColor™ Protein Loading Buffer Pack	1000 samples	R1011
10X Tris-glycine-SDS Electrophoresis Buffer	1 liter	B46
10X Tris-glycine Electrophoresis Buffer	1 liter	B47
10X Tris-tricine-SDS Electrophoresis Buffer	1 liter	B48
Loading Buffer Pack	2000 samples	R0891

References

1. Rabilloud, T. et al., Silver-staining of proteins in polyacrylamide gels: a general overview, *Cell. Mol. Biol.*, 40, 57-75, 1994. *Cell. Mol. Biol.* 40, 57-75, 1994.
2. Blum, H., Beier, H. And Gross, H.J., Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels, *Electrophoresis*, 8, 93-99, 1987.

Trademarks

PageSilver, PageRuler and PageBlue are trademarks of Fermentas.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

SAFETY INFORMATION

PageSilver™ Silver Staining Kit

The following kit components are hazardous:

Developing Reagent



Xi Irritant

Risk phrases

R36 Irritating to eyes.

Safety phrases

- S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S60 This material and its container must be disposed of as hazardous waste.

Staining Reagent



Xi Irritant



N Dangerous for the environment

Risk phrases

- R36/38 Irritating to eyes and skin.
R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

- S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S37 Wear suitable gloves.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S57 Use appropriate container to avoid environmental contamination.
S60 This material and its container must be disposed of as hazardous waste.

Formaldehyde



T Toxic

Hazard-determining components of labeling:

Formaldehyde

Methanol

Risk phrases

- R23/24/25 Toxic by inhalation, in contact with skin and if swallowed.
- R34 Causes burns.
- R37 Irritating to respiratory system.
- R40 Limited evidence of a carcinogenic effect.
- R43 May cause sensitization by skin contact.
- R68/20/21/22 Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed.

Safety phrases

- S4 Keep away from living quarters.
- S7/9 Keep container tightly closed and in a well-ventilated place.
- S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
- S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
- S60 This material and its container must be disposed of as hazardous waste.