INSTRUCTIONS

Pierce™ Color Silver Stain Kit

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24597

Number	Description
24597	**Pierce Color Silver Stain Kit**, sufficient reagents to stain 25 (18cm^2) or 40 (10 × 13cm) gels

Kit Contents:

Silver Reagent, 500mL

Reducer Aldehyde Reagent, 500mL

Reducer Base Reagent, 500mL

Stabilizer Base Reagent, 500mL

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Introduction

The Thermo Scientific Pierce Color Silver Stain Kit enables highly sensitive and versatile staining of proteins electrophoresed in 1D and 2D polyacrylamide gels. Generally, bands containing less than 1ng of protein are detectable with this silver stain. Proteins stain in reproducibly distinct colors visible against an amber background. Nucleic acids, as in the products of PCR, that have been electrophoresed in polyacrylamide gels also may be stained using this kit.[1,2]

Several slightly different silver staining methods were developed in the early 1980s. All methods depend on the kinetics of impregnation, reduction of silver ions to elemental silver and its complexation to protein nucleation sites in a porous gel matrix.[3] The Pierce Color Silver Stain Kit uses a weakly acidic solution of silver nitrate for gel impregnation, followed by development in formaldehyde at alkaline pH.[4,5] After incubation in the Stabilizer Base Working Solution, silver nitrate and formaldehyde are either reacted or diffused from the gel.

When a stained gel is viewed over a bright white light source, several distinct colors may be recognized. Five basic colors commonly visible in protein samples with the Pierce Color Silver Stain are black, blue, brown, red and yellow. Color differences correspond to differences in protein amino acid composition, although detailed predictions are not possible. Nevertheless, color patterns are reproducible for a given sample and staining time and temperature, and they may be used to distinguish overlapping proteins and other subtleties in 2D systems.[5]

Regardless of gel size and thickness, the Pierce Color Silver Stain Kit is well characterized and supported by this complete procedure for obtaining excellent staining results for routine and special electrophoresis experiments involving polyacrylamide gels.

Important Product Information

- Use clean glass or polyethylene trays. Wash utensils and trays thoroughly, and rinse away soaps with dilute nitric acid or Thermo Scientific PCC-54 Detergent (Product No. 72288).

- Use high quality reagents and ultrapure water to prepare and electrophorese gels. Water must be free of organics, heavy metals, carbonates, and bacteria.
- Prepare reagents and perform entire procedure at room temperature (25°C). Increasing reagent temperature by 5°C above room temperature may significantly increase background (darkness) and reduce sensitivity of the procedure. Control of time and temperature is essential for optimal sensitivity and reproducibility.
- Use a platform shaker to ensure efficient and uniform washes and staining during the procedure.
- The stained gel will have an amber-colored background and may appear dark in normal room lighting. View the protein bands over a bright light source, such as a white fluorescent light box. Gel results may be photographed using these same lighting conditions.

Additional Materials Required

- Glass or polyethylene trays
- Platform shaker
- Ultrapure water
- Ethanol
- Acetic Acid

Procedure

A. Fix and Wash Gel

1. Fix (18cm × 18cm) gel overnight in fixative buffer (50% Ethanol + 5% Acetic Acid) or for 4 hours with frequent changes of the fixative buffer. Smaller gels (10cm × 13cm) usually require only 1-2 hours for fixation. Fixation is complete when the gel ceases to shrink. Alternatively, fixation may be accomplished using 2.5% (w/v) glutaraldehyde for 1.5 hours.[6] Use high purity glutaraldehyde (49%) to minimize background.
2. Wash gel with four changes of ultrapure water, as described in Table 1. Total wash time may be decreased if the number of washes is increased. Gel is ready to be stained when it returns to its original size.

Table 1. Wash times for gels of different thickness.

Gel Thickness (mm)	Water Washes (min)				Total Time (min)
0.125	5	5	5	5	20
0.500	15	15	15	15	60
0.750	30	30	30	30	120
1.000	40	40	40	40	160
1.500	45	45	45	45	180

B. Prepare Stain Reagent Working Solutions (WS)

Prepare Stain Reagent Working Solutions as directed in Table 2. Prepare Silver Working Solution and Reducer Working Solutions immediately before use. The volumes of reagents indicated are sufficient to stain two large-format (18cm^2) gels. To stain multiple gels in the same tray or different size gels, change the reagent volumes but not their concentrations.

Table 2. Preparation of Working Solutions (WS).

Silver Working Solution	add 20mL of Silver Reagent to 280mL water
Reducer Aldehyde Working Solution	add 20mL of Reducer Aldehyde Reagent to 130mL water
Reducer Base Working Solution	add 20mL of Reducer Base Reagent to 130mL water
Stabilizer Base Working Solution*	add 20mL of Stabilizer Base Reagent to 880mL water

* The Stabilizer Working Solution may be prepared in large quantities stored at room temperature indefinitely.

C. Stain Gel

1. Incubate gel in Silver WS for recommended time (Table 3).
2. Water rinse gel for the few seconds recommended (Table 3).

3. Prepare Reducer WS by combining equal volumes of Reducer Aldehyde WS and Reducer Base WS immediately before use. Incubate gel in Reducer WS for the few minutes recommended (Table 3).
4. Water rinse gel only if ≥ 1.0mm thick or the gel background is dark.
5. Incubate gel in Stabilizer WS for the recommended time (Table 3).

   Note: Do not leave the gel in Stabilizer WS for more than 2 hours, or excessive gel swelling will occur.

Table 3. Recommended reaction times for gels of different thickness.

Gel Thickness (mm)	Silver WS (min)	Water Rinse	Reducer WS* (min)	Water Rinse	Stabilizer WS (min)	Total Time (min)
0.125	5	0	0.25-0.5	0	15	21
0.500	15	3-5 seconds	1-2	0	30	47
0.750	30	5-10 seconds	3-5	0	30	65
1.000	30	20 seconds	5	5 seconds	40	80
1.500	20	20-30 seconds	9-10	15 seconds	60	90

*Reducer WS consists of equal volume mixture of Reducer Aldehyde WS and Reducer Base WS. Prepare Reducer WS immediately before use.

D. Gel Storage and Drying

- For wet storage, wrap gel in clear plastic wrap or place it in a resealable bag.
- Dry gels as follows: (1) Add 5% glycerol to the stabilizer base solution and soak gel for approximately 1 hour. (2) Remove gel from and place between two sheets of cellophane membrane or filter paper backing. (3) For a 1.5mm gel, use a gel dryer with temperature between 50-90°C for 1-2 hours. For 0.75-1.00mm thick gels, air-dry the gel in a plastic frame.

Troubleshooting

Problem	Possible Cause	Solution
Precipitate forms during Stabilizer step	Excess silver on gel or in residual solution	Use water rinse after Silver Stain step
Dark background	Silver binding with reducer caused a dark background	Water rinse after Reducer step
Dark bands	Excess protein in gel	Decrease protein load in gel
Clear bands (ghost bands)	Silver Stain did not stain certain proteins	Use Thermo Scientific GelCode Blue Stain Reagent (Product No. 24590)
Black or light colored gel	Solutions expired	Purchase new kit
Vertical streaks in gel	Contaminants in running buffer	Prepare buffers and solutions from quality reagents and ultrapure water
Horizontal streaks	Degradation products of 2-mercaptoethanol (2-ME)	Filter 2-mercaptoethanol before use or use a different reducing agent in sample loading buffer
Light background or no staining	Protein washed off during rinse step	Decrease water rinse time after Reducer step

Related Products

24612 **Pierce Silver Stain Kit**

LC6060 **SimpleBlue™ SafeStain**

24615	**Imperial™ Protein Stain**
24582	**E-Zinc™ Reversible Stain Kit**
XP04200BOX	**Novex™ Tris-Glycine protein gels** (see thermofisher.com/proteingels for a complete listing)
NW04120BOX	**Bolt™ Bis-Tris Plus protein gels** (see thermofisher.com/proteingels for a complete listing)

Cited References

1. Baege, A., *et al.* (2002). Cervical epithelial cells transduced with the papillomavirus E6/E7 oncogenes maintain stable levels of oncoprotein expression but exhibit progressive, major increases in hTERT gene expression and telomerase activity. *Am J Pathol* **160**:1251-7.
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3. Rabilloud, T. (1990). Mechanisms of protein silver staining in polyacrylamide gels: a 10-year synthesis. *Electrophoresis* **11**:785-94.
4. Sammons, D.W., *et al.* (1981). Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. *Electrophoresis* **2**:135-41.
5. Sammons, D.W., *et al.* (1984). Applicability of color silver stain (GelCode System) to protein mapping with two-dimensional gel electrophoresis. In: Two dimensional gel electrophoresis of proteins. Academic Press: New York.
6. Slisz, M.L. and Van Frank, R.M. (1985). Enhanced sensitivity of GelCode Silver Staining by glutaraldehyde fixation of proteins in two-dimensional electrophoresis. *Electrophoresis* **6**:405-7.

Product Reference

Hase, M.E., *et al*. (2001). Amino acid substitutions of coiled-coil protein Tpr abrogate anchorage to the nuclear pore complex but not parallel, in-register homodimerization. *Mol Biol Cell* **12**: 2433-52.

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