INSTRUCTIONS

Pierce Silver Stain Kit

MAN0016358
Rev. A.0
Pub. Part No. 2161478.6

24612

Number	Description
24612	**Pierce Silver Stain Kit**, contains sufficient reagents to stain 20 mini gels
	Kit Contents:
	Silver Stain Sensitizer, 2mL
	Silver Stain Enhancer, 25mL
	Silver Stain, 500mL
	Silver Stain Developer, 500mL
	Storage: Upon receipt store at room temperature.

Introduction

The Thermo Scientific™ Pierce™ Silver Stain Kit is a rapid and ultrasensitive silver stain system for protein detection in polyacrylamide gels. The stain performs consistently and reliably for both first-time and experienced users for high, low or gradient percentage gels in single-dimension or 2-D format.

The Pierce Silver Stain Kit provides consistency, high sensitivity, low background and a flexible protocol. Careful manufacturing procedures and thorough quality testing ensure that the kit yields excellent results with every use, independent of the polyacrylamide gel type or buffer system being used. Most proteins are easily detectable at low nanogram or subnanogram amounts, and background levels are very low (see Additional Information section at the end of these instructions). The protocol clearly indicates how both fixing and staining steps can be extended overnight without compromising performance, a feature that enhances convenience for ordinary workday schedules. Likewise, the default 30-minute staining step can be decreased to 5 minutes, enabling the staining procedure to be completed in less than 20 minutes after gel fixation.

Procedure Summary

1. Wash gel 2 × 5 minutes in ultrapure water.
2. Fix gel 2 × 15 minutes in 30% ethanol:10% acetic acid solution.
3. Wash gel 2 × 5 minutes in 10% ethanol, then 2 × 5 minutes in ultrapure water.
4. Prepare Sensitizer Working Solution (50µL Sensitizer with 25mL water).
5. Sensitize gel for 1 minute, then wash 2 × 1 minute with water.
6. Prepare Stain Working Solution (0.5mL Enhancer with 25mL Stain).
7. Stain gel for 30 minutes.
8. Prepare Developer Working Solution (0.5mL Enhancer with 25mL Developer).
9. Wash gel 2 × 20 seconds with ultrapure water, then develop gel for 2-3 minutes until bands appear.
10. Stop with 5% acetic acid for 10 minutes.

Pierce Biotechnology
3747 N. Meridian Road
PO Box 117
Rockford, IL 61105 USA
(815) 968-0747
(815) 968-7316 fax
Thermofisher.com

Additional Materials Required

- Ethanol, ~50mL
- Acetic Acid, ~25mL
- Ultrapure water, ~1L

Procedure for Silver Staining Proteins in Polyacrylamide Gels

Important Notes:

- Perform all steps in a single clean staining tray (plastic or glass) with constant gentle shaking.
- Throughout the procedure, use sufficient volumes of solution to thoroughly cover the gel. Generally, 25mL is sufficient for a mini gel in small tray. Use a generous volume for wash steps.
- Avoid using metal utensils throughout the procedure. Use a clean, plastic spatula or gloved hands to manipulate the gel. When using gloved hands, touch the gel only at the edges to avoid depositing protein on the surface, which may cause background.
- Fixing, Ethanol and Stop solutions (used in steps 2, 3 and 10) may be prepared in advance. Other solutions must be prepared immediately before use.
- The Silver Stain Enhancer is used in both Stain and Developer Working Solutions. Do not use Stain or Developer directly without first adding the Enhancer (steps 7 and 9) immediately before use.

1. Wash gel in ultrapure water for 5 minutes. Replace the water and wash for another 5 minutes.
2. Fix gel in 30% ethanol:10% acetic acid solution (i.e., 6:3:1 water:ethanol:acetic acid) for 15 minutes. Replace the solution and fix for another 15 minutes.

   Note: Gel may be kept in fixing solution overnight without affecting stain performance.
3. Wash gel in 10% ethanol solution for 5 minutes. Replace solution and wash for another 5 minutes.
4. Wash gel in ultrapure water for 5 minutes. Replace water and wash for another 5 minutes.
5. Prepare Sensitizer Working Solution by mixing 1 part Silver Stain Sensitizer with 500 parts ultrapure water (e.g., mix 50µL Sensitizer with 25mL water).
6. Incubate gel in Sensitizer Working Solution for exactly 1 minute, then wash with two changes of ultrapure water for 1 minute each.
7. Prepare Stain Working Solution by mixing 1 part Silver Stain Enhancer with 50 parts Silver Stain (e.g., 0.5mL of Enhancer with 25mL Stain).
8. Incubate gel in Stain Working Solution for 30 minutes.

   Note: Gel may be incubated in Stain Working Solution for as short as 5 minutes or as long as overnight without affecting stain performance.
9. Prepare Developer Working Solution by mixing 1 part Silver Stain Enhancer with 50 parts Silver Stain Developer (e.g., mix 0.5mL of Enhancer with 25mL Developer).
10. Prepare 5% acetic acid solution as a Stop Solution.
11. Quickly wash gel with two changes of ultrapure water for 20 seconds each.
12. Immediately add Developer Working Solution and incubate until protein bands appear (2-3 minutes).

    Note: Protein bands will begin to appear within 30 seconds and then continue to develop. Between 2 and 3 minutes, protein detection vs. background is optimal. After 3 minutes, lane background signal may increase to undesirable levels.
13. When the desired band intensity is reached, replace Developer Working Solution with prepared Stop Solution (5% acetic acid). Wash gel briefly, then replace Stop Solution and incubate for 10 minutes.

Troubleshooting

Problem	Possible Cause	Solution
Bands faint or not visible	Insufficient development	Develop gel for >5 minutes or add newly prepared Developer Working Solution
	Minimal or no protein present in sample	Check protein concentration in the original sample
	Improper solution preparation or skipped steps	Check solution preparation and follow procedure
	Excessive water wash before development step	Wash gel 3 × 10 minutes with ultrapure water, then repeat staining procedure shortening this wash step
High background	Stained gel was overdeveloped	Reduce development time
	Washing step(s) was missed or poor quality water was used	Do not skip or reduce wash steps. Use ultrapure water
	Contaminated equipment was used	Use clean equipment rinsed with ultrapure water
	Impure chemical was used for gel preparation or precast gel has expired	Use analytical grade chemicals or use precast gels that have not expired
	Stop solution was not effective in halting development of gel	Prepare new 5% acetic acid and replace it twice in the first minutes of incubation with the gel

Additional Information

A. Visit our web site for additional information about this product, including the following item:

- Tech Tip #50: Process stained polyacrylamide gels for mass spectrometry

B. Example gels stained with the Pierce Silver Stain Kit

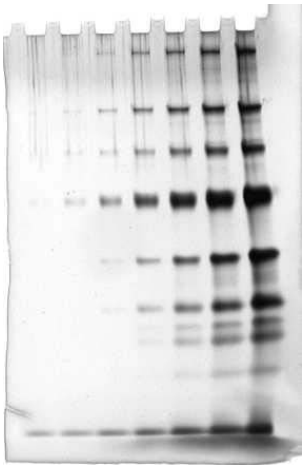

Figure 1. 4-20% Precise™ Gel containing two-fold serial dilutions of purified proteins. The third band from the top is BSA, with the rightmost lane containing 14ng.

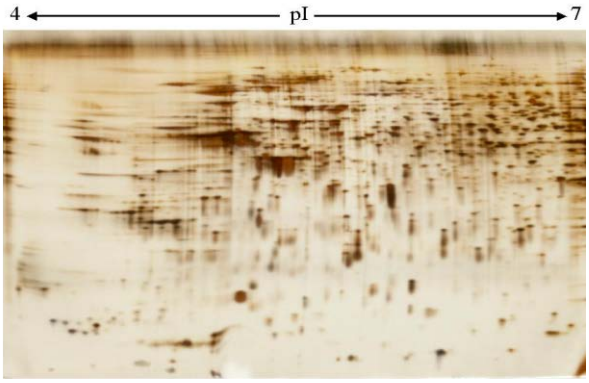

Figure 2. 2-D gel of 15µg of TNFα-induced HeLa cytosolic extract prepared with the Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Kit (Product No. 78833).

C. Staining Performance in Different Gels

Table 1. Relative performance (sensitivity and low background) of gel sources and buffer systems with the Pierce Silver Stain Kit. A five-star rating is optimal. In these Pierce experiments, Bio-Rad Tris-tricine gels performed poorly by comparison to other gels but still yielded acceptable results for routine protein detection.

Gel Supplier	Gel Buffer System	Performance Rating
Thermo Scientific Precise Gels	Tris-HEPES (gradient and homogeneous)	★★★★★
Invitrogen	Tris-Glycine (homogeneous and gradient)	★★★★★
	Bis-Tris	★★★★★
	Tris-Tricine	★★★
Bio-Rad	Tris•HCl	★★★
	Tris-Tricine	★★
	Criterion™ Gels (1D and 2-D gels)	★★★★
Homemade Gels	12% Tris-Glycine	★★★★★

Related Thermo Scientific Products

24614	**Silver Stain Rescue Reagent**, 40mL, sufficient to reduce background on 50-100 mini gels
24597	**Color Silver Stain Kit,** sufficient to stain 25 (18cm^2) or 40 (10 × 13cm) 2-D gels
24620	**PageBlue™ Protein Staining Solution,** 1L
24615	**Imperial™ Protein Stain,** 1L, coomassie R-250 stain sufficient for 50 (8 × 10cm) mini gels
24582	**E-Zinc™ Reversible Stain Kit,** sufficient to stain 20 (8 × 10cm) mini gels
26616	**PageRuler™ Prestained Protein Ladder**
26634	**Spectra™ Multicolor Broad Range Protein Ladder**
XP00080BOX	**Novex™ WedgeWell™ 8% Tris-Glycine Mini Gels, 10-well**