INSTRUCTIONS

Pierce™ Silver Stain for Mass Spectrometry

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24600

Number	Description
24600	**Pierce Silver Stain for Mass Spectrometry,** sufficient reagents to stain 20 mini gels and destain > 500 gel pieces for subsequent elution and analysis by mass spectrometry

Kit Contents:

Silver Stain Sensitizer, 2mL

Silver Stain Enhancer, 25mL

Silver Stain Developer, 500mL

Silver Stain, 500mL

Silver Destain Reagent A, 4mL

Silver Destain Reagent B, 14mL

Storage: Upon receipt store at ambient temperature.

Introduction

The Thermo Scientific Pierce Silver Stain for Mass Spectrometry is a complete kit for rapid and ultra-sensitive silver staining of proteins in polyacrylamide gels and efficient destaining of excised gel pieces for mass spectrometry analysis. This kit enables both first-time and experienced users to achieve consistent and reliable staining using high, low and gradient percentage gels in single-dimension and 2D formats. The optimized staining method ensures extremely sensitive staining while minimizing covalent crosslinking of protein to the gel matrix, which can inhibit protein recovery. The destaining reagents facilitate complete removal of silver from stained protein bands and maximum protein recovery for subsequent mass spectrometry analysis.

Procedure for Protein Staining 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 a 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 gel. When using gloved hands, touch only the gel edges to avoid depositing protein on the surface, which may cause background.
- 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.

A. Additional Materials Required

- Fixing Solution: 30% ethanol, 10% acetic acid (i.e., 6:3:1 water:ethanol:acetic acid)
- Stop Solution: 5% acetic acid
- Ethanol Wash: 10% ethanol

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B. Procedure

1. Wash gel in ultrapure water for 5 minutes. Replace the water and wash for another 5 minutes.
2. Decant water and add Fixing Solution to the gel. Incubate for 15 minutes at room temperature. Replace solution and fix for another 15 minutes. Gel may remain in fixing solution overnight without affecting stain performance.
3. Wash gel with the Ethanol Wash 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. Just before use, 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. Mix 1 part Silver Stain Enhancer with 100 parts Silver Stain (e.g., mix 0.25mL of enhancer with 25mL stain) and immediately add it to the gel. Incubate gel for 5 minutes.
8. Prepare developer working solution by mixing 1 part Silver Stain Enhancer with 100 parts Silver Stain Developer (e.g., mix 0.25mL of enhancer with 25mL developer).
9. Quickly wash gel with two changes of ultrapure water for 20 seconds each.
10. Immediately add developer working solution and incubate until protein bands appear (2-3 minutes).

    Note: Protein bands begin to appear within 30 seconds and continue to develop. Protein detection vs. background is optimal from 2 to 3 minutes. After 3 minutes, lane background signal may increase to undesirable levels.
11. When the desired band intensity is reached, replace developer working solution with Stop Solution. Wash gel briefly, then replace acetic acid and incubate for 10 minutes.
12. Immediately proceed to excising and destaining the gel pieces procedure.

Procedure for Excising and Destaining Polyacrylamide Gel Pieces

A. Additional Materials Required

- Wash Solution: 25mM ammonium bicarbonate, 50% acetonitrile. Store at 4°C.
- Light box
- Scalpel or spot picker

B. Procedure

1. Wash gel in ultrapure water for 10 minutes. Replace water and wash for another 10 minutes.
2. While using a light box to illuminate the gel, excise protein band with a clean scalpel or spot picker.
3. From a blank region of the gel, excise another gel piece of the same size to use as a control sample.
4. Place gel pieces in clean 0.5mL microcentrifuge tubes.
5. Prepare destain solution by combining 74µL of Silver Destain Reagent A, 245µL of Silver Destain Reagent B and 4mL of ultrapure water, which is sufficient to treat 10 gel pieces. Use this solution within the same day; do not store for prolonged periods.
6. Add 0.2mL of the destain solution to the gel pieces, mix gently and incubate at room temperature for 15 minutes.
7. Remove the destain solution. Incubate gel pieces in 0.2mL of additional destain solution for 15 minutes.
8. Remove the destain solution and wash gel pieces three times for 10 minutes each with 0.2mL of Wash Solution.
9. Proceed with in-gel trypsin digestion or other protein elution steps in preparation for the desired mass spectrometry method (see Additional Information and Related Thermo Scientific Products sections). Alternatively, store the gel pieces overnight at -20°C. Do not exceed overnight storage.

Troubleshooting

Problem	Possible Cause	Solution
Bands faint or not visible	Insufficient development time	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 three times for 10 minutes each to completely remove the previous solutions and redo the staining procedure starting at step 2. Do not over wash prior to incubation in the developer
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 and 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

C. Preparing Samples for Mass Spectrometry

After using the Silver Stain Kit, proteins can be recovered and processed by several methods for mass analysis. For in-gel trypsin digestion, with or without reduction and alkylation, use the Thermo Scientific In-Gel Tryptic Digest Kit (Product No. 89871). Consider following the recommendations of the core facility that will be performing the MS analysis.

D. Example Mass Spectrometry Results

Several proteins were processed using the Pierce Silver Stain for Mass Spectrometry (Table 1). For each protein, 50ng was separated by gel electrophoresis and stained. Protein bands were excised and destained according to the protocol. Proteins were trypsinized and eluted using the In-Gel™ Tryptic Digest Kit (Product No. 89871) and then analyzed by MALDI/MS.

Table 1. MALDI/MS peptide fragment analysis of silver-stained proteins. Protein samples were either untreated or reduced and alkylated before trypsinization.

	Untreated		Reduced/Alkylated	
Protein	# of Peptides Observed	% Coverage*	# of Peptides Observed	% Coverage*
BSA	63	21	42	56
Ovalbumin	40	13	47	23
Chymotrypsinogen A	47	9	33	27
Myoglobin	32	19	24	43

*The percent of protein sequence that was identified.

Related Products

89871 **In-Gel Tryptic Digest Kit**

87782 **Pierce™ C18 Tips, 10µL bed**

24615 **Imperial™ Protein Stain**, 1L, coomassie R-250 stain

NW04120BOX **Bolt™ Bis-Tris Plus protein gels** (see thermofisher.com/proteingels for a complete listing)

24614 **Pierce Silver Stain Rescue Reagent**, 40mL, sufficient to reduce background on 50-100 mini gels

26619 **PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa**

For research use only. Not for use in diagnostic procedures.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Current product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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