

PageBlue™ Protein Staining Solution

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24620

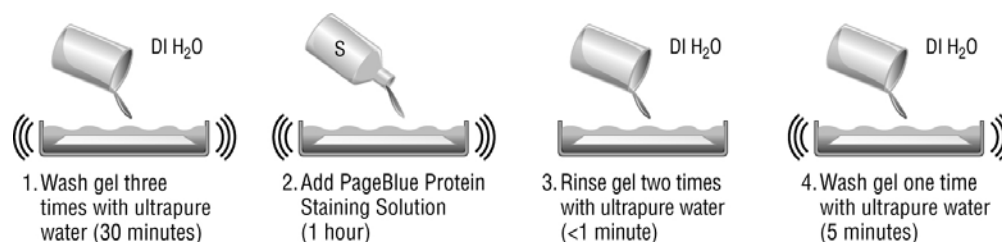
Number	Description
24620	PageBlue Protein Staining Solution, 1L, sufficient reagents for up to 150 mini gels

Storage: Upon receipt store product at room temperature. Product shipped at ambient temperature.

Introduction

The Thermo Scientific PageBlue Protein Staining Solution uses the colloidal properties of coomassie G-250 dye for protein staining on polyacrylamide gels and polyvinylidene fluoride (PVDF) membranes. Simple procedures and excellent sensitivity provide the researcher with an efficient staining method and a dynamic range of 5-500ng, which is ~10 times more sensitive than traditional coomassie R-250-based dyes. A unique reagent, PageBlue Protein Staining Solution stains only proteins and allows bands to be viewed directly on the gel.

Procedure Summary



Important Product Information

- PageBlue Protein Staining Solution can be reused up to three times without noticeable loss in detection sensitivity.
- Large or multiple gels will require added staining solution to be submersed; adjust volumes accordingly.
- Fixing gel proteins with 25% isopropanol/10% acetic acid solution or 12% trichloroacetic acid (TCA) for 15 minutes can increase staining sensitivity.
- The first wash step in the following procedures is designed to remove sodium dodecyl sulfate (SDS) from the gel, because SDS interferes with staining. Native gels do not contain SDS and, therefore, this step can be omitted from native PAGE applications.
- Small proteins are susceptible to leaching from the gel during the staining procedure and may require fixation before staining the gel (See Additional Information Section).

Procedure for Staining Gels

1. Place gel in a clean tray and wash three times for 10 minutes each using 100-200mL of ultrapure water with gentle agitation.
2. Discard last wash. Add 20mL, or sufficient volume to cover the gel, of PageBlue Protein Staining Solution and incubate at room temperature for 60 minutes with gentle agitation.

Note: Gels may be stained overnight without increasing the background.

- Discard the staining solution and rinse the gel two times with ultrapure water.

Note: The staining solution can be saved and reused up to three additional times.

- Wash the gel for 5 minutes with 100-200mL of ultrapure water.

Note: Washing the gel for a longer period or frequently replacing the water will enhance sensitivity. Additionally, placing a folded Kimwipes™ Tissue in the container to absorb excess dye will accelerate the destaining process.

Alternative Microwave Procedure for Staining Gels

Note: The microwave procedure results in faster staining with equivalent sensitivity (5ng) to the standard procedure.

- Place gel in an uncovered microwaveable tray containing 100mL of ultrapure water and microwave for 60 seconds. Remove tray from the microwave, gently agitate for 4 minutes and discard water. Repeat two additional times.
- Discard the last wash and add 20mL of PageBlue Protein Staining Solution, or sufficient volume to cover the gel, and microwave on high power for 20-30 seconds. Do not allow the reagent to boil.
- Gently agitate the gel in the staining solution for an additional 20 minutes.
- Discard staining solution and rinse the gel two times with ultrapure water.
- Wash the gel for 5 minutes with 100-200mL of ultrapure water.

Note: Washing the gel for a longer period or frequently replacing the water will enhance sensitivity. Additionally, placing a folded Kimwipes Tissue in the container to absorb excess dye will accelerate the destaining process.

Procedure for Staining PVDF Membranes

Note: The PVDF membrane must be completely dry before staining.

- Place the dried PVDF membrane in a tray containing a sufficient amount of PageBlue Protein Staining Solution to cover the membrane. Gently agitate the tray for 2 minutes.
- Discard staining solution and wash the membrane in 30% ethanol for 5 minutes with gentle agitation.
- To completely remove stain, wash the membrane in a 30% acetonitrile/20% ethanol solution for 5 minutes with gentle agitation.

Troubleshooting

Problem	Possible Cause	Solution
High background	SDS interfered with staining	Increase the number of washes and/or wash volumes before staining
		Destain the gel for 5 minutes with 25% isopropanol/10% acetic acid solution or 12% trichloroacetic acid
No band development	No protein was present in sample	Load a known amount of purified protein as a control
	Insufficient amount of protein in sample	Load more total protein
	SDS was not completely removed from gel	Wash gel more extensively before staining
	Problems with transfer	Confirm that the transfer buffer and transfer conditions are correct
High background on membrane	Insufficient destaining time	Destain the membrane in 30% acetonitrile/20% ethanol solution for an additional 5 minutes

Additional Information

A. Procedure for Preparing Small Proteins for Gel Staining

Note: Small proteins (< 10kDa) require fixation with glutaraldehyde before staining the gel with PageBlue Protein Staining Solution. Other common protein fixatives (e.g., acetic acid, isopropanol, ethanol, TCA, etc.) are not suitable for this purpose, as proteins will be washed out of the gel during the staining procedure.

1. Place gel in a clean tray and add 100mL of ultrapure water to the gel. Wash for 1 minute with gentle agitation.
2. Discard the wash and add 50mL, or sufficient volume to cover the gel, of new 5% glutaraldehyde solution.
3. Fix for 30 minutes with gentle agitation.
4. Discard fixation solution and rinse gel three times for 5 minutes each with 100-200mL of ultrapure water and gentle agitation.
5. Proceed directly to the Procedure for Staining Gels or the Alternative Microwave Procedure for Staining Gels.

B. Information Available on Our Website

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

Related Products

26630	PageRuler™ Unstained Broad Range Protein Ladder, 2 × 250μL
26632	PageRuler Unstained Low Range Protein Ladder, 2 × 250μL
26614	PageRuler Unstained Protein Ladder, 2 × 250μL
26623	Spectra™ Multicolor Broad Range Protein Ladder, 10 × 250μL
26634	Spectra Multicolor Broad Range Protein Ladder, 2 × 250μL
24615	Imperial™ Protein Stain, 1L
24617	Imperial Protein Stain, 3 × 1L
24612	Pierce Silver Stain Kit
24600	Pierce Silver Stain Kit for Mass Spectrometry
LC2676	Novex™ Tris-Glycine SDS Sample Buffer (2X)
LC2675	Novex Tris-Glycine SDS Running Buffer (10X)
XP04200BOX	Novex Tris-Glycine protein gels (see thermofisher.com/proteingels for a complete listing)

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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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