INSTRUCTIONS

Pierce™ 6xHis Protein Tag Stain Reagent Set

24570

0870.3

Number	Description
24570	**Pierce 6xHis Protein Tag Stain Reagent Set,** contains sufficient reagent to stain 10 mini gels
	Contents:
	6xHis Protein Tag Stain, 500mL
	6xHis Protein Tag Developer, 500mL
	Storage: Upon receipt store components at room temperature. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce 6xHis Protein Tag Stain specifically stains His-tagged proteins electrophoresed in polyacrylamide gels (SDS-PAGE). Upon staining, fusion proteins expressing polyhistidine tags will fluoresce yellow when exposed to UV light. Because no fixing steps are required, staining with this kit does not inhibit subsequent total protein staining with Thermo Scientific GelCode Blue Stain Reagent or electrophoretic transfer to membrane.

The polyhistidine tag, consisting of six consecutive histidine residues (hence 6xHis), has several advantages over other types of tags; it is relatively small, poorly immunogenic, and usually does not interfere with native protein folding. Furthermore, 6xHis-tagged proteins are easily purified by immobilized metal-ion affinity chromatography (IMAC) and may be detected on Western blots using monoclonal anti-His antibodies, Ni-NTA (nickel-nitrilotriacetic acid) conjugates or Thermo Scientific HisProbe-HRP (nickel-chelated horseradish peroxidase; see Related Thermo Scientific Products).

The Pierce 6xHis Protein Tag Stain adds to the repertoire of 6xHis tag applications by allowing specific detection directly in gels. No Western blotting step is required to confirm proper expression and purification of His-tagged proteins.

Procedure for 6xHis-Tagged Protein Staining

Procedural Notes:

- This system requires at least 0.2µg of a 35kDa (5.7pmol) His-tagged protein per band for detection with a CCD camera and at least 2µg (57pmol) His-tagged protein per band for detection with a UV transilluminator.
- Detection requires illumination of the stained gel with UV-light at a wavelength in the range 280-310nm.
- Wash the gel thoroughly (Step 1) before staining; residual SDS in the gel prevents binding of stain.
- Bis-Tris gels run in MOPS or MES buffer may require fixing in 50% methanol:7% acetic acid for 15 minutes before performing the stain procedure. After electrophoresis, fix the gel and then proceed with Step 1 of the procedure.

Staining Procedure

1. After electrophoresis, wash the gel by gently agitating with 100mL of ultrapure water for 20 minutes. Repeat this step twice. Remove the water wash.
2. Add 50mL of 6xHis Protein Tag Stain to the gel and gently agitate for 5 minutes.
3. Wash gel with 100mL of ultrapure water for 15 minutes. Repeat this step once. Remove the water wash.
4. Add 50mL of 6xHis Protein Tag Developer to the gel and agitate for 15 minutes.
5. Wash gel with 100mL of ultrapure water for 15 minutes. Repeat this step once.

6. Irradiate gel with ultraviolet light (~300nm) using a UV illumination box (transilluminator) or a CCD camera. 6xHis-tagged proteins will fluoresce as yellow bands when viewed under ultraviolet light.

   Note: The fluorescence signal is stable for several hours in gels stored in water. Signal may be detectable, if somewhat attenuated, after overnight storage.

7. 6xHis-stained gels may be directly stained for total protein using GelCode™ Blue Stain Reagent or other general protein stains without intermediate processing. Alternatively, stained gels may be equilibrated in appropriate buffer and transferred to nitrocellulose or PVDF membrane for subsequent staining or Western blotting.

Troubleshooting

Problem	Possible Cause	Solution
No experimental protein detected, but positive controls not used for comparison	Troubleshooting was difficult without data on positive and negative controls	Use positive and negative controls
No bands detected for either positive control of experimental protein	Poor quality or insufficient exposure to appropriate UV-light source	If possible, use a CCD camera for detection; ensure that UV lamp delivers the appropriate wavelength for excitation (280-310nm)
	Experimental protein is poorly expressed (insufficient loading)	Insufficient protein electrophoresed per lane for the detection method used
	Insufficient washing; residual SDS in gel prevents binding of stain	Wash gel for 3 × 20 minutes in ultrapure water and restain
	Experimental protein is small (< 20kDa) and diffused from gel during washing step	Fix the gel 50% methanol:7% acetic acid for 15 minutes before performing the water wash
	Poor diffusion of stain into gel	Increase staining time to 10 minutes (step 2) — this may be repeated on the same gel
Positive control detected but not experimental protein	Experimental protein not expressed at sufficient levels in the lysate being tested	Load more lysate per lane or otherwise check that the target protein is expressed at all
	Experimental recombinant protein is not tagged with 6xHis	Check for presence of tag by an independent method (e.g., detection or purification by nickel-chelate chemistry)
	6xHis tag on experimental protein is blocked by interfering substances in sample	Verify that nickel and other 6xHis-binding reagents were not brought forward from a previous step and use only high-quality water
Non-tagged proteins detected	Weak cross-reaction staining of proteins containing histidine clusters	Wash gel for additional time in water (step 5)
		Slightly decrease staining time (step 2)
		Adjust exposure time and other settings to minimize weak, non-specific staining

Related Thermo Scientific Products

24590 **GelCode Blue Stain Reagent**, 500mL

24570 **Pierce 6xHis Protein Tag Stain Reagent Set**

15168 **SuperSignal™ West HisProbe™ Kit**

15165 **HisProbe-HRP**

78100 **B-PER™ 6xHis Fusion Protein Column Purification Kit**

78300 **B-PER 6xHis Fusion Protein Spin Purification Kit**

78994 **Y-PER™ 6xHis Fusion Protein Column Purification Kit**

15442 **Nickel Coated Plates**, 96-well clear plates (white and black also available)

15143 **Copper Coated, High Binding Capacity Plates,** 96-well clear plates (white and black also available)

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”).

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer’s exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.