INSTRUCTIONS

KryptonTM Protein Stain

46628	46629	46630
-------	-------	-------

Number	Description	
46628	Krypton Protein Stain (10X), 20 ml, sufficient reagent to stain up to 4 mini gels	
46629	Krypton Protein Stain (10X), 100 ml, sufficient reagent to stain up to 20 mini gels	
46630	Krypton Protein Stain (10X), 500 ml, sufficient reagent to stain up to 100 mini gels	
	Excitation Wavelength: 520 nm	
	Emission Wavelength: 580 nm	

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

Thermo Scientific Krypton Protein Stain enables sensitive fluorescent staining of proteins separated by 1-D or 2-D SDS-PAGE. The stain is sensitive down to 0.25 ng using the standard protocol, or down to 2 ng using the rapid 30 minute protocol. The Krypton Protein Stain is supplied as a 10X stock solution that is diluted with water before use. This proteinspecific stain allows band visualization using a variety of fluorescence imaging systems. The optimal imaging systems are laser-based fluorescence scanners capable of exciting and detecting at 520 nm and 580 nm, respectively; however, filteredbased CCD camera systems are also effective. The Krypton Protein Stain has a linear quantitative range of three to four orders of magnitude and is compatible with mass spectrometry analysis.

Important Product Information

- For best results, equilibrate the Krypton Protein Stain to room temperature and dilute to 1X immediately before use. The 1X stain solution may be stored up to seven days at 4°C with minimal loss of sensitivity. A white cloudy precipitate may form when diluting cold 10X Krypton Protein Stain, but the precipitate rapidly dissolves when the 1X solution is mixed.
- Use sufficient volumes of stain, fixing and destain solutions to completely cover the gel and allow it to float freely. Typically, 35-50 ml is sufficient for an 8 × 8 cm gel and 75-100 ml is sufficient for a 13 × 9 cm gel.

Additional Materials Required

- Gel Fixing Solution: 40% ethanol (v/v), 10% (v/v) acetic acid in ultrapure water
- Destaining Solution: 5% (v/v) acetic acid in ultrapure water

Standard Procedure for Staining Gels

The standard procedure allows band visualization in ~2 hours, 40 minutes with sensitivity down to 0.25 ng.

- 1. Equilibrate the Krypton Protein Stain (10X) to room temperature and dilute 10-fold with ultrapure water. Prepare just enough reagent for the gel(s) being stained.
- 2. Remove the gel(s) from the gel cassette or plates. Place gel in a clean tray with a sufficient volume of Gel Fixing Solution to immerse the gel. Cover the tray, and place it on a rocker or shaker and gently agitate for 30 minutes.
- 3. Decant the fixing solution. Add more fixing solution and agitate gently for another 30 minutes.
- 4. Carefully decant the fixing solution. To remove residual solution from the gel, add ultrapure water to the tray and agitate the gel for 5 minutes.
- 5. Carefully decant the water and add a sufficient volume of 1X Krypton Protein Stain to immerse gel. Cover the tray with aluminum foil to minimize light exposure. Place tray on a shaker and agitate gel for 1 hour. Staining for 2 hours to overnight may improve band development for some proteins.

<u>18</u>29.0





- 6. Carefully decant the stain solution. Add the Destaining Solution, cover the tray and agitate gently for 5 minutes.
- 7. Remove the Destaining Solution and replace with an equal volume of ultrapure water. Gently agitate for 15 minutes.
- 8. Carefully decant water and replace it with more ultrapure water. Agitate the gel gently for 15 minutes.
- 9. For best results, detect bands using visible laser-based imagers equipped with a 532 nm laser light source. Although the optimum emission filter is 580 nm, 600 nm filters are also compatible. The gel can be imaged on any platform with the respective excitation and emission filters.

Rapid Procedure for Staining Gels

The rapid procedure allows band visualization in 30 minutes with sensitivity down to 2 ng.

- 1. Equilibrate the Krypton Protein Stain (10X) to room temperature and dilute 10-fold with ultrapure water. Prepare just enough reagent for the gel(s) being stained.
- 2. Remove the gel(s) from the gel cassette or plates. Place gel in a clean tray with a sufficient volume of Gel Fixing Solution to immerse the gel. Cover the tray, place it on a rocker or shaker and gently agitate for 5 minutes.
- 3. Decant the fixing solution from the tray and add new fixing solution. Gently agitate tray for 5 minutes.
- 4. Carefully decant the fixing solution. To remove residual solution from the gel, add ultrapure water to the tray and agitate the gel for 1 minute.
- 5. Carefully decant the water and add a sufficient volume of 1X Krypton Protein Stain to immerse the gel. Cover the tray with aluminum foil to minimize light exposure. Place tray on a shaker and gently agitate for 15 minutes.
- 6. Carefully decant the staining solution. Add a sufficient volume of ultrapure water and gently agitate for 3 minutes.
- 7. For best results, detect bands using visible laser-based imagers equipped with a 532 nm laser light source. Although the optimum emission filter is 580 nm, 600 nm filters are also compatible. The gel can be imaged on any platform with the respective excitation and emission filters.

Troubleshooting

Problem	Possible Cause	Solution
Bands or spots are not visible	Imaging system malfunction	Check instrument manual for troubleshooting
	No proteins in the gel	Verify that there is protein in the gel by staining with another method (e.g., Imperial TM Protein Stain, Product No. 24615)
	Wrong filter sets selected	Check excitation and emission settings to confirm the correct filter sets are being used

Related Thermo Scientific Products

28398BupH™ Tris-HEPES-SDS Running Buffer Packs, 10 packs28378BupH™ Tris-Glycine-SDS Running Buffer Packs, 40 packs89871In-Gel Tryptic Digestion Kit898652-D Sample Prep for Soluble Proteins Kit898662-D Sample Prep for Insoluble Proteins Kit24615Imperial™ Protein Stain, 1 L24612SilverSNAP® Stain Kit II	25200-25244	Precise™ Protein Gels, see catalog or web site for a complete listing
28378BupH™ Tris-Glycine-SDS Running Buffer Packs, 40 packs89871In-Gel Tryptic Digestion Kit898652-D Sample Prep for Soluble Proteins Kit898662-D Sample Prep for Insoluble Proteins Kit24615Imperial™ Protein Stain, 1 L24612SilverSNAP® Stain Kit U	28398	BupH [™] Tris-HEPES-SDS Running Buffer Packs, 10 packs
89871In-Gel Tryptic Digestion Kit898652-D Sample Prep for Soluble Proteins Kit898662-D Sample Prep for Insoluble Proteins Kit24615Imperial™ Protein Stain, 1 L24612SilverSNAP® Stain Kit II	28378	BupH [™] Tris-Glycine-SDS Running Buffer Packs, 40 packs
898652-D Sample Prep for Soluble Proteins Kit898662-D Sample Prep for Insoluble Proteins Kit24615Imperial™ Protein Stain, 1 L24612SilverSNAP® Stain Kit II	89871	In-Gel Tryptic Digestion Kit
898662-D Sample Prep for Insoluble Proteins Kit24615Imperial™ Protein Stain, 1 L24612SilverSNAP® Stain Kit II	89865	2-D Sample Prep for Soluble Proteins Kit
24615Imperial™ Protein Stain, 1 L24612SilverSNAP® Stain Kit II	89866	2-D Sample Prep for Insoluble Proteins Kit
24612 SilverSNA P [®] Stain Kit II	24615	Imperial [™] Protein Stain, 1 L
	24612	SilverSNAP [®] Stain Kit II

U.S. Patent Pending on Krypton[™] Protein Stain Technology



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 versions of product instructions are available at <u>www.thermo.com/pierce</u>. For a faxed copy, call 800-874-3723 or contact your local distributor. © 2010 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.