INSTRUCTIONS

Krypton™ Infrared Protein Stain

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

53070 53071 53072

1892.0

Number	Description
53070	**Krypton™ Infrared Protein Stain (10X),** 20 ml, sufficient reagent to stain up to 4 mini gels
53071	**Krypton™ Infrared Protein Stain (10X),** 100 ml, sufficient reagent to stain up to 20 mini gels
53072	**Krypton™ Infrared Protein Stain (10X),** 500 ml, sufficient reagent to stain up to 100 mini gels
	Excitation Wavelength: 690 nm
	Emission Wavelength: 718 nm

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

Introduction

The Krypton™ Infrared Protein Stain enables sensitive fluorescent visualization of proteins separated by 1-D or 2-D SDS-PAGE. The Krypton™ Infrared Protein Stain is supplied as a 10X solution that is diluted with ultrapure water before use. This reagent is specific for staining proteins and allows band visualization with a variety of infrared fluorescence imaging systems. Imaging is obtained using laser-based fluorescence scanners capable of exciting and detecting at 680 nm and 720 nm, respectively, or filtered-based CCD camera systems. Krypton™ Infrared Protein Stain is sensitive to 0.25 ng using the standard protocol or down to 1 ng using the rapid 60 minute protocol. The stain has an excellent dynamic range and is compatible with subsequent analysis by mass spectrometry.

Important Product Information

- For best results, dilute the Krypton™ Infrared Protein Stain to 1X immediately before use. The 1X stain solution may be stored for up to seven days at 4°C with minimal loss of sensitivity.
- 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

- Acetic Acid, reagent grade
- Ethanol, reagent grade
- 10% Tween®-20 (Surfact-Amps® 20, Product No. 28320)

Material Preparation

1X Krypton™ Infrared Protein Stain	Prepare 1X Krypton™ Infrared Protein Stain by diluting the 10X solution 10-fold with ultrapure water.
Gel Fixing Solution	50% Ethanol (v/v), 15% (v/v) acetic acid in ultrapure water
Destaining Solution	5% (v/v) acetic acid, 0.1% (v/v) Tween®-20 in ultrapure water

Standard Procedure for Staining Gels

The standard procedure allows band visualization in ~1.75 hours to overnight with sensitivity down to 0.25 ng.

1. 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 10 minutes. Two alternative fixation solutions may be used: 40% ethanol, 10% acetic acid; or 15% methanol, 15% ethanol, 50 mM sodium hydroxide.
2. Decant the Gel Fixing Solution. Add more fixing solution and agitate gently for another 10 minutes.
3. 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.
4. Carefully decant the water and add a sufficient volume of 1X Krypton™ Infrared Protein Stain to immerse the gel. Cover the tray with aluminum foil to minimize exposure to light. Place tray on a shaker and agitate gel for 1 hour at room temperature. Staining for 2 hours to overnight may improve band development for some proteins.
5. Carefully decant the stain solution. Add the Destaining Solution, cover the tray and agitate gently for 5 minutes. Two alternative destaining solutions include 5% acetic acid or ultrapure water.
6. Remove the Destaining Solution and replace with an equal volume of ultrapure water. Gently agitate for 10 minutes at room temperature.
7. Carefully decant water and replace it with more ultrapure water. Agitate gently for an additional 10 minutes.
8. The primary fluorescent excitation maximum of Krypton™ Infrared Protein Stain is 690 nm. The fluorescence emission maximum is at 718 nm. The most sensitive results are obtained using infrared laser-based imagers equipped with a 680 nm laser light source. The preferred emission filter is 720 nm. The gel can also be imaged on any imaging platform with the respective excitation and emission filters.

Rapid Procedure for Staining Gels

The rapid procedure allows band visualization in ~60 minutes with sensitivity down to 1 ng.

1. Remove the gel(s) from the gel cassette or plates. Place gel in a clean tray with sufficient volume of Gel Fixing Solution to immerse the gel. Cover the tray, place it on a rocker or shaker, and agitate gently for 10 minutes.
2. Decant the Gel Fixing Solution from the tray and add new fixing solution. Agitate gently for 10 minutes.
3. Carefully decant the Gel Fixing Solution. To remove residual solution from the gel, add ultrapure water to the tray and agitate the gel for 5 minutes.
4. Carefully decant the water and add a sufficient volume of 1X Krypton™ Infrared Protein Stain to immerse the gel. Cover the tray with aluminum foil to minimize light exposure. Place tray on a shaker and agitate gel for 15 minutes at room temperature.
5. Carefully decant the stain solution and add sufficient volume of Destaining Solution. Cover the tray and agitate gently for 5 minutes.
6. Carefully decant the Destaining Solution and replace with an equal volume of ultrapure water. Gently agitate for 7 minutes at room temperature.
7. Carefully decant the water and replace with new ultrapure water. Agitate gently for an additional 7 minutes.
8. The primary fluorescent excitation maximum of Krypton™ Infrared Protein Stain is 690 nm. The fluorescence emission maximum is at 718 nm. The most sensitive results are obtained using infrared laser-based imagers equipped with a 680 nm laser light source. The preferred emission filter is 720 nm. The gel can also be imaged on any imaging 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
	There are no proteins in the gel	Verify that there is protein in the gel by staining with another method (e.g., Imperial™, SilverSNAP® or GelCode™ Blue Stains)
	Wrong filter sets were selected	Check excitation and emission settings to confirm the correct filter sets are being used
Spots and streaks visible	Contamination of running buffers, sample loading buffer, fixing solutions or water; or contamination of imager surface	Use newly prepared solutions with ultrapure water and thoroughly clean the surface of the gel imager
	Gels were handled with bare hands or powder-coated gloves	Always handle gels with powder-free gloves

Related Pierce Products

25200-25244 **Precise™ Protein Gels,** see catalog or website for a complete listing

46628 **Krypton™ Protein Stain (10X),** 20 ml, for fluorescent staining gels

46629 **Krypton™ Protein Stain (10X),** 100 ml, for fluorescent staining gels

46630 **Krypton™ Protein Stain (10X),** 500 ml, for fluorescent staining gels

89871 **In-Gel Tryptic Digestion Kit**

89865 **2-D Sample Prep for Soluble Proteins Kit**

89866 **2-D Sample Prep for Insoluble Proteins Kit**

24615 **Imperial™ Protein Stain,** 1 L

24612 **SilverSNAP® Stain Kit II**

28320 **Surfact-Amps® 20,** 6 × 10 ml

* U.S. Patent Pending on Krypton Infrared Protein Stain Technology

Tween® is a registered trademark of ICI Americas.

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