INSTRUCTIONS

Krypton™ Glycoprotein Staining Kit

PIERCE
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53074

1891.0

Number	Description
53074	**Krypton™ Glycoprotein Staining Kit,** sufficient material to stain 10 mini gels

Kit Contents:

Krypton™ Staining Reagent, 300 µl dye solution in PBS, store at -20°C

Staining Buffer, 250 ml, store at room temperature

Oxidizing Reagent, 2.5 g, sufficient to make 250 ml, store at room temperature

Positive Control (Horseradish Peroxidase), 1 mg, store at 4°C

Negative Control (Soybean Trypsin Inhibitor), 1 mg, store at 4°C

Storage: Upon receipt store components as indicated. Product is shipped at ambient temperature.

Introduction

The Krypton™ Glycoprotein Stain enables fast and sensitive fluorescent staining for the detection of glycoproteins. The stain reacts with periodate-oxidized carbohydrate groups on glycoproteins, creating a bright red-fluorescent signal. The stain is sensitive down to 15 ng of glycoprotein per band, depending upon the nature and degree of glycosylation on the proteins. This glycoprotein-specific 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 654 nm and 673 nm, respectively; however, filter-based CCD camera systems are also effective. After staining with Krypton™ Glycoprotein Stain, the gel can be stained with a total-protein stain, such as Krypton™ Protein Stain. The Krypton™ Glycoprotein Stain has a linear quantitative range of approximately three orders of magnitude and is compatible with mass spectrometry analysis.

Additional Materials Required

- Tris-glycine polyacrylamide gel
- Ethanol
- Glacial acetic acid
- Sodium hydroxide
- Ultrapure water
- Aluminum foil

Material Preparation

Fixing Solution	Mix 400 ml of ethanol, 300 ml of glacial acetic acid and 300 ml of ultrapure water. Store at room temperature.
Wash Solution	Mix 150 ml glacial acetic acid with 850 ml ultrapure water. Store at room temperature.
3% Acetic Acid	Mix 30 ml glacial acetic acid with 970 ml ultrapure water. Store at room temperature.
Destaining Solution	Dissolve 3 g of sodium hydroxide in 1 L of ultrapure water to make 75 mM. Store at room temperature.
Oxidizing Solution	Add 250 ml of 3% acetic acid to the Oxidizing Reagent and mix until it is completely dissolved. Store at room temperature.
Positive Control (Horseradish Peroxidase)	Reconstitute vial contents with 1 ml of ultrapure water to make 1 mg/ml. Immediately before use, dilute stock solution to 50 µg/ml with SDS-PAGE sample buffer and apply 500 ng (10 µl) per lane. Aliquot the 1 mg/ml stock solution into single-use volumes and store at -20°C.
Negative Control (Soybean Trypsin Inhibitor)	Reconstitute vial contents with 1 ml of ultrapure water to make 1 mg/ml. Immediately before use, dilute stock solution to 50 µg/ml with SDS-PAGE sample buffer and apply 500 ng (10 µl) per lane. Aliquot the 1 mg/ml stock solution into single-use volumes and store at -20°C.
Krypton™ Staining Reagent	After using the Krypton™ Staining Reagent for the first time, make 30 µl aliquots of the reagent, cover the tubes with aluminum foil and store at -20°C.

Procedure

- Use sufficient volumes of solutions to completely cover the gel. The gel must float freely during all steps of the procedure. Typically, 25-30 ml is sufficient for an 8 × 8 cm gel and 75-80 ml is sufficient for a 13 × 9 cm gel.
- Perform the procedure in a fume hood.
- For incubations in the dark, cover the tray containing the gel with aluminum foil.

A. Fixing the Gel

1. After separating the proteins by standard SDS-PAGE, place the gel into a suitable container. Rinse the gel five times with ultrapure water.
2. Add the Fixing Solution and incubate the gel at room temperature (RT) for 30 minutes with mild agitation. Repeat this step once.
3. Discard the Fixing Solution. Add Wash Solution and incubate the gel at RT for 10 minutes with mild agitation. Repeat this step once.

B. Carbohydrate Oxidation and Staining

Note: Warm the Krypton™ Staining Reagent to RT before opening. Always prepare the Stain Working Solution just before use and do not store any remaining Stain Working Solution.

1. Add the Oxidizing Solution to the gel and incubate at RT in the dark for 20 minutes with mild agitation.
2. Discard the Oxidizing Solution. Add Wash Solution and incubate the gel at RT for 10 minutes with mild agitation. Repeat this step once.
3. Mix 25 µl of Krypton™ Staining Reagent with 25 ml of Staining Buffer to prepare the Stain Working Solution.
4. Add the Stain Working Solution to the gel and incubate at RT in the dark for 60 minutes with and mild agitation. For 2-D gels, incubate for 1.5-2 hours.

C. Destaining the Gel

1. Rinse the gel twice with ultrapure water. Incubate the gel in ultrapure water at RT for 10 minutes in the dark with mild agitation.
2. Add the Destaining Solution to the gel and incubate at RT for 20 minutes in the dark with mild agitation. Repeat this step once. DO NOT extend the destaining time as this will decrease sensitivity.

D. Detecting Glycoproteins

1. The Krypton™ Staining Reagent has an excitation/emission maxima of ~654/673 nm. For best results, detect glycoprotein bands using visible laser-based imagers equipped with a 633 nm laser light source. The gel can be imaged on any platform with the appropriate excitation and emission filters. Using the appropriate filters is necessary to obtain maximum sensitivity.
2. To store the gel after detection, incubate the gel in 3% acetic acid at RT and mild agitation for 5 minutes. Store the gel in ultrapure water at RT in the dark.

E. Staining the Gel for Total Proteins

After staining with Krypton™ Glycoprotein Stain, the gel can be stained with a total protein stain, such as Krypton™ Protein Stain (Product No. 46629). Before staining with Krypton™ Protein Stain perform the following steps.

1. Add 3% acetic acid to the gel and incubate at RT in the dark for 5 minutes with mild agitation.
2. Discard the acetic acid. Add ultrapure water to the gel and incubate at RT in the dark for 10 minutes with mild agitation.
3. Follow the procedure described in the instructions for the Krypton™ Protein Stain.

Troubleshooting

Problem	Possible Cause	Solution
Bands or spots not visible	Imaging system malfunction	Check instrument manual for trouble shooting
	No proteins in the gel	Verify that there is protein in the gel by staining with another method, such as Krypton™ Protein Stain (Product No. 46629)
	Wrong filter sets selected	Check excitation and emission settings to confirm the correct filter sets are being used

Related Pierce Products

46629 **Krypton™ Protein Stain (10X),** 100 ml

53071 **Krypton™ Infrared Protein Stain (10X),** 100 ml

28398 **BupH™ Tris-Glycine-SDS Running Buffer Packs**

89871 **In-Gel Tryptic Digestion Kit**

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

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

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