

INSTRUCTIONS

GelCode[®] Glycoprotein Staining Kit

24562



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

0855w

Number	Description
24562	GelCode[®] Glycoprotein Staining Kit , contains sufficient materials to stain 10 mini gels or 20 nitrocellulose membranes (8 x 8 cm)

Kit contents:

GelCode[®] Glycoprotein Stain, 250 ml

Oxidizing Reagent, 2.5 gm, sufficient to make 250 ml of Oxidizing Solution

Reducing Reagent, 1.25 gm, sufficient to make 250 ml of Reducing Solution

Positive Control (Horseradish Peroxidase), 1 mg

Negative Control (Soybean Trypsin Inhibitor), 1 mg

Storage: Upon arrival store all kit components at 4°C. This kit is shipped at ambient temperature.

This product is guaranteed for one year from the date of purchase when handled and stored properly.

Introduction

Glycoproteins are widely distributed in nature and serve a variety of functions. They are found in blood, secretions, cell membranes and connective tissues. Glycoproteins consist of carbohydrate moieties covalently linked to a polypeptide backbone. These diverse proteins may have a carbohydrate component that represents <1% to >80% of the total weight. Sugars that commonly occur in glycoproteins include galactose, mannose, glucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, sialic acid, fucose and xylose.

The GelCode[®] Glycoprotein Staining Kit will detect glycoprotein sugar moieties in polyacrylamide gels and on nitrocellulose membranes. When treated with Oxidizing Reagent (periodic acid), glycols present in glycoproteins are oxidized to aldehydes. After completing the procedure, the glycols are stained, yielding magenta bands with a light pink or colorless background.

Note: Please read this entire instruction booklet before staining.

Procedure for Staining Glycoproteins in SDS-Polyacrylamide Gels

Additional Materials Required

- Methanol, Spectroscopy Grade
- Acetic Acid, Glacial

Material Preparation

- **3% Acetic Acid:** Mix 30 ml of glacial acetic acid with 970 ml of ultrapure water. Store at room temperature (RT).
- **50% Methanol:** Mix 250 ml of methanol with 250 ml of ultrapure water. Store at RT.
- **Oxidizing Solution:** Add 250 ml of 3% acetic acid to the bottle labeled "Oxidizing Reagent" and mix until material is completely dissolved. Store the solution at RT.
- **Reducing Solution:** Add 250 ml of ultrapure water to the bottle labeled "Reducing Reagent" and mix until material is completely dissolved. Store the solution at RT.

Telephone: 800-8-PIERCE (800-874-3723) or 815-968-0747 • Fax: 815-968-7316 or 800-842-5007
www.piercenet.com • Customer Service: cs@piercenet.com • Technical Assistance: ta@piercenet.com

- **Horseradish Peroxidase Positive Control:** Reconstitute vial contents with 0.5 ml of ultrapure water to produce a 2 mg/ml solution. Dilute to a 1 mg/ml solution with the sample buffer for SDS-PAGE analysis. For 8 x 8 cm gels, apply 5 µl or 10 µl of reconstituted positive control per lane. After reconstitution of Horseradish Peroxidase Positive Control, aliquot and store at -20°C.
- **Soybean Trypsin Inhibitor Negative Control:** Reconstitute vial contents with 0.5 ml of ultrapure water to produce a 2 mg/ml solution. Dilute to a 1 mg/ml solution with the sample buffer for SDS-PAGE analysis. For 8 x 8 cm gels, apply 5 µl or 10 µl of reconstituted negative control per lane. After reconstitution of Soybean Trypsin Inhibitor Negative Control, aliquot and store at -20°C.
- **Sample Dilution:** Dilute sample to be analyzed to a 1 mg/ml solution with the sample buffer for SDS-PAGE analysis. For 8 x 8 cm gels, apply 5 µl or 10 µl of diluted sample per lane.

Procedure

Note: Perform the following steps in a fume hood.

1. After electrophoresis, fix gel by completely immersing it in 100 ml of 50% methanol for 30 minutes.
2. Wash gel by gently agitating with 100 ml of 3% acetic acid for 10 minutes. Repeat this step once.
Note: This can be a stopping point. The gel can be left in water overnight at 4°C.
3. Transfer gel to 25 ml of Oxidizing Solution and gently agitate for 15 minutes.
4. Wash gel by gently agitating with 100 ml of 3% acetic acid for 5 minutes. Repeat this step two additional times.
5. Transfer gel to 25 ml of GelCode[®] Glycoprotein Staining Reagent and gently agitate for 15 minutes.
Note: If crystals form in the GelCode[®] Glycoprotein Stain, centrifuge solution and remove the supernatant for use. DO NOT HEAT to dissolve crystals. Only use stain after crystals have been removed.
6. Transfer gel to 25 ml of Reducing Solution and gently agitate for 5 minutes.
7. Wash gel extensively with 3% acetic acid and then with ultrapure water. Glycoproteins appear as magenta bands. Store gel in 3% acetic acid.

Procedure for Staining Glycoproteins on Nitrocellulose Membranes

A. Additional Materials Required

- **Acetic Acid, Glacial**

B. Material Preparation

- **3% Acetic Acid:** Mix 15 ml of glacial acetic acid with 485 ml of ultrapure water. Store at room temperature (RT).
- **Oxidizing Solution:** Add 250 ml of 3% acetic acid to the bottle labeled “Oxidizing Reagent” and mix until material is completely dissolved. Store solution at RT.
- **Reducing Solution:** Add 250 ml of ultrapure water to the bottle labeled “Reducing Reagent” and mix until material is completely dissolved. Store solution at RT.

Procedure

Note: Perform the following steps in a fume hood.

1. Wash membrane by gently agitating with 20 ml of 3% acetic acid for 10 minutes. Repeat this step once.
2. Transfer membrane to 10 ml of Oxidizing Solution and gently agitate for 15 minutes.
3. Wash membrane by gently agitating with 10 ml of 3% acetic acid for 5 minutes. Repeat this step two more times.
4. Transfer membrane to 10 ml of GelCode[®] Glycoprotein Staining Reagent and gently agitate for 15 minutes.

Note: If crystals form in the GelCode[®] Glycoprotein Stain, centrifuge solution and remove the supernatant for use. DO NOT HEAT to dissolve crystals. Only use stain after crystals have been removed.

5. Transfer membrane to 10 ml of Reducing Solution and gently agitate for 5 minutes.
6. Wash membrane extensively with 3% acetic acid and then with ultrapure water. Glycoproteins appear as magenta bands. Store membrane in 3% acetic acid.

Related Pierce Products

Number	Description
23260	Glycoprotein Carbohydrate Estimation Kit , sufficient reagents for 250 microplate assays or 60 standard test tube assays
23259	Lyophilized Glycoprotein Standards Set , includes negative and positive controls
24590	GelCode[®] Blue Stain Reagent , for the total protein stain of 25 SDS-PAGE mini gels

© Pierce Biotechnology, Inc., 9/2002. Printed in the USA.