Pierce™ Glycoprotein Staining Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Pierce™ Glycoprotein Staining Kit detects 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.

Contents and storage

The Pierce™ Glycoprotein Staining Kit is shipped at ambient temperature and contains sufficient materials to stain 10 mini-gels or 20 nitrocellulose membranes (8 cm × 8 cm).

Component	Amount	Storage
Glycoprotein Stain [1]	250 mL	Store all kit components at 4°C upon receipt
Oxidizing Reagent [2]	2.5 g (Sufficient for 250 mL of Oxidizing Solution)	
Reducing Reagent	1.25 g, (Sufficient for 250 mL of Reducing Solution)	
Positive Control (Horseradish Peroxidase)	1 mg	
Negative Control (Soybean Trypsin Inhibitor)	1 mg	

[1] To comply with Department of Transportation (DOT) shipping regulations, the Glycoprotein Stain is shipped in a separate package from the remaining components. Upon receipt of both packages, components may be placed together in a single kit box for storage.

[2] Oxidizing Reagent may contain liquid droplets.

Procedure for staining glycoproteins in polyacrylamide gels

Required materials not supplied

- Methanol, spectroscopy grade
- Acetic acid, glacial

Prepare staining solutions

Prepare reagents for polyacrylamide gel staining.

Reagent	Action	Storage
3% Acetic Acid	Mix 30 mL of glacial acetic acid with 970 mL of ultrapure water.	Store at room temperature.
50% Methanol	Mix 250 mL of methanol with 250 mL of ultrapure water.	
Oxidizing Solution	Add 250 mL of 3% acetic acid to the bottle labeled "Oxidizing Reagent", then mix until material is completely dissolved.	
Reducing Solution	Add 250 mL of ultrapure water to the bottle labeled "Reducing Reagent", then mix until material is completely dissolved.	
Horseradish Peroxidase Positive Control	Add 0.5 mL of ultrapure water to reconstitute contents of vial at a final concentration of 2 mg/mL.	Store at –20°C as aliquots after reconstitution.
Soybean Trypsin Inhibitor Negative Control	Add 0.5 mL of ultrapure water to reconstitute contents of vial at a final concentration of 2 mg/mL.	

Prepare controls and samples

1. Dilute reconstituted controls to 1 mg/mL with SDS-PAGE sample buffer.
2. Dilute samples to be analyzed to a 1 mg/mL with SDS-PAGE sample buffer.
3. For 8 × 8 cm gels, apply 5 µL or 10 µL of the diluted control or sample in the appropriate lane, then perform electrophoresis.

Stain the gel

Note: Perform the following steps in a fume hood.

1. After electrophoresis, immerse the gel completely in 100 mL of 50% methanol and fix for 30 minutes.
2. Immerse the gel in 100 mL of 3% acetic acid, then wash with gentle agitation 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 the gel to 25 mL of Oxidizing Solution and gently agitate for 15 minutes.
4. Wash the gel by gently agitating with 100 mL of 3% acetic acid for 5 minutes. Repeat this step two more times.

5. Transfer the gel to 25 mL of Glycoprotein Stain and gently agitate for 15 minutes.

   Note: If crystals form in the Glycoprotein Stain, centrifuge the solution and use the supernatant. DO NOT heat to dissolve crystals. Only use stain after crystals have been removed.
6. Transfer the gel to 25 mL of Reducing Solution and gently agitate for 5 minutes.
7. Wash the 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

Required materials not supplied

- Acetic Acid, Glacial

Prepare staining solutions

Prepare reagents for nitrocellulose membrane staining.

Reagent	Action	Storage
3% Acetic Acid	Mix 15 mL of glacial acetic acid with 485 mL of ultrapure water.	Store at room temperature.
Oxidizing Solution	Add 250 mL of 3% acetic acid to the bottle labeled "Oxidizing Reagent", then mix until material is completely dissolved.	
Reducing Solution	Add 250 mL of ultrapure water to the bottle labeled "Reducing Reagent", then mix until material is completely dissolved.	

Stain the membrane

Note: Perform the following steps in a fume hood.

1. Immerse the membrane in 20 mL of 3% acetic acid, then wash with gentle agitation for 10 minutes. Repeat this step once.
2. Transfer the membrane to 10 mL of Oxidizing Solution and gently agitate for 15 minutes.
3. Wash the membrane by gently agitating with 10 mL of 3% acetic acid for 5 minutes. Repeat this step two more times.
4. Transfer the membrane to 10 mL of Glycoprotein Stain and gently agitate for 15 minutes.

   Note: If crystals form in the Glycoprotein Stain, centrifuge the solution and remove the supernatant for use. DO NOT heat to dissolve crystals. Only use stain after crystals have been removed.
5. Transfer the membrane to 10 mL of Reducing Solution and gently agitate for 5 minutes.
6. Wash the membrane extensively with 3% acetic acid and then with ultrapure water. Glycoproteins appear as magenta bands. Store membrane in 3% acetic acid.

Accessory products

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 mini-gels
24594	GelCode™ Blue Safe Protein Stain,1L
24580	Pierce™ Reversible Protein Stain Kit for Nitrocellulose Membranes
24602	Pierce™ Silver Stain Kit
24597	Pierce™ Color Silver Stain Kit
28378	BupH™ Tris-Glycine-SDS Buffer Packs, 40 packs
25200-44	Precise™ Protein Gels, see catalog or website for a complete listing
28398	BupH™ Tris-HEPES-SDS Buffer Packs, 10 packs
89871	In-Gel Tryptic Digestion Kit

Limited product warranty

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