G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Glycoprotein Staining Kit

With Rapidstain™ for Enhanced Glycoprotein & Non Glycoproteins Staining

(Cat. # 786-254)

INTRODUCTION

Glycoprotein Staining kit detects glycoprotein sugar in gel electrophoresis matrix and on nitrocellulose membranes. The kit uses an enhanced Periodic Acid-Schiff (PAS) method for detection of glycoprotein sugars. The supplied oxidizing agent first oxidizes the cis-diol sugar groups to aldehydes. The aldehyde groups react with the sensitive Glyco-Stain Solution forming Schiff bonds and producing strong magenta color bands.

In addition to glycoprotein staining, the kit is supplied with RAPIDstain™, an enhanced Coomassie stain. RAPIDstain™ can be used after glycoprotein staining to detect non-glycosylated proteins and the use of the stain enhances glycoprotein staining.

The Glycoprotein Staining kit is highly convenient as all the key reagents required for staining are supplied and a unique positive & negative control is included. In addition, the kit allows for the detection of glycosylated and non-glycosylated proteins on a single gel or membrane. Suitable for 10 mini gels (8 x 8cm) or 20 nitrocellulose membrane (8 x 8cm).

ITEM(S) SUPPLIED (Cat. # 786-254)

Description	Size
Glyco-Stain Solution	250ml
Glyco-Oxidizing Reagent	For 250ml
Glyco-Reducing Regent	For 250ml
Glyco-Positive & Negative Control	100µg
RAPIDstain™	250ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the Glyco-Positive & Negative Control at -20°C and all other components at 4°C.

ADDITIONAL ITEMSREQUIRED

- Glacial Acetic Acid
- Methanol

SENSITIVITY

Detection limit is dependent on extent of glycosylation, protein size amount of glycoprotein present and the nature of sugars that are oxidized to aldehyde.

PREPARATION BEFORE USE:

1. **Washing Solution I (3% acetic acid):** Mix 30ml glacial acetic acid in 970ml deionized water.
2. **Washing Solution II (50% methanol):** Mix 250ml methanol in 250ml deionized water.
3. **Glyco-Stain Solution:** Before use transfer an appropriate amount of Glyco-Stain to a 15 or 50ml centrifuge tube. If crystal are present then centrifuge at 1000 x g for 5 minutes and use the cleared supernatant for staining. Do not use Glyco-Stain with crystals for staining or warm the solution to dissolve the crystals.
4. **Glyco-Oxidizing Reagent:** Add 250ml of Washing Solution I directly to the Glyco-Oxidizing Reagent bottle. Mix to dissolve the dry oxidizing agent present in the bottle. Store the solution at room temp.
5. **Glyco-Reducing Reagent:** Add 250ml of deionized water directly to the Glyco-Reducing Regent bottle. Mix to dissolve the dry reducing agent present in the bottle. Store the solution at room temp.
6. **Glyco-Positive and Negative controls:** Before opening the tube, centrifuge the tube at 15,000xg for 5 minutes. Suspend the proteins in 105µl SDS loading buffer; incubate at room temperature for 15 minutes with periodic mixing (vortex). Aliquot the reconstituted control in to 10µl aliquots and store at -20°C. Use 10µl, 1 aliquot, for each control lane.

PROTOCOL

Staining glycoprotein in SDS polyacrylamide gels

1. After electrophoresis remove the gel from electrophoresis cassette.
2. Fixing: Rinse gel in 100ml Washing Solution II for 30 minutes. Discard the solution.
3. Wash: Wash gel with 100ml of Washing Solution I for 10 minutes. Discard the wash. Repeat this wash step once.
4. Oxidation: Add 25ml of Glyco-Oxidizing Reagent. Gently agitate for 15 minutes.
5. Wash: Wash gel with 100ml of Washing Solution I for 5 minutes. Discard the wash. Repeat this wash step twice.
6. Staining: Add 25ml Glyco-Stain Solution. Agitate gently for 15 minutes. Discard the stain.
7. Reduction: Add 25ml Glyco-Reduction Reagent. Gently agitate for 5 minutes.
8. Wash gel three times with 100ml Washing Solution I for 10 minutes each wash, and then rinse with deionized water.
9. Glycoproteins are seen as magenta bands. Store gel in Washing Solution I or in drying solution (Cat. # 786-685) for drying the gel.
10. Glycoprotein Staining Controls: Appear as a cluster of positive bands or Glycoproteins (magenta bands) around 40-80kD and non-glycoprotein bands that will be visible only (as blue bands) when treated with RAPIDstain™.

Visualization of non-glycosylated proteins and enhancement of glycoprotein staining

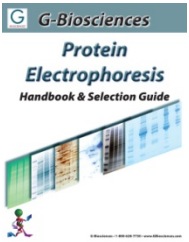
1. After staining of the glycoproteins the gel may be stained with RAPIDstain™. RAPIDstain™ will stain unglycosylated proteins blue and enhance the visualization of the stained glycoprotein.
2. Wash gel three times with deionized water, 10 minutes each wash.
3. Develop the gel with 25ml RAPIDstain™ for 5-40 minutes. Do not stain for >40 minutes.
   NOTE: *Monitor the development of the stain and remove from the RAPIDstain™ once a suitable level of staining has been achieved.*
4. Wash the gel in deionized water for 30 minutes.
5. For long term storage, store in Washing Solution I.

Staining glycoproteins on nitrocellulose membranes

1. Wash: Wash gel with 20ml of Washing Solution I for 10 minutes. Discard the wash. Repeat this wash step once.
2. Oxidation: Add 10ml Glyco-Oxidizing Reagent to the membrane and gently agitate for 15 minutes. Discard the Oxidizing solution.
3. Wash: Wash the membrane with 10ml Wash Solution I for 5 minutes. Discard the wash. Repeat this wash step twice.
4. Staining: Add 10ml Glyco-Stain Solution and gently agitate 15 minutes. Discard the stain.
5. Reduction: Transfer the membrane into 10ml Glyco-Reducing Reagent and gently agitate for 5 minutes.
6. Wash: Wash gel 3-5 times with 50ml Washing Solution I for 10 minutes each wash, and then rinse with deionized water.
7. Glycoproteins are seen as magenta bands. Store membrane in Wash Solution I.

RELATED PRODUCTS

Download our Protein Electrophoresis Handbook.



hhttp://info.gbiosciences.com/complete-protein-electrophoresis-handbook/

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