PRODUCT INFORMATION

Glycoprotein Staining Kit (Cat# PSG-0025)

Product Information for PSG-0025:

Introduction:
Traditional Schiff's reagent requires microgram amounts of protein for detection. At the same time they are carbohydrate specific, which means that non-glycosylated proteins are not stained. Glycoprotein Staining kit is silver enhancement of traditional staining methods -it combines dye staining with silver staining which results in at least two fold increase in sensitivity for detection of proteoglycans and glycoproteins. Glycoprotein Staining kit stains both glycosylated and non-glycosylated proteins. The kit is enough for 25 mini gels.

Content	
Glyco-Fixing Solution [5X]	250 ml
Glyco-Oxidizing Reagent	1 vial (14g)
Glyco-Reducing Regent	1 vial (7g)
GlycoBlue Solution [50X]	25 ml
Glyco-Positive Control (Horseradish Peroxidase)	2.5µg
Glyco-Negative Control (Soybean Trypsin Inhibitor)	2.5µg

Supplied with Mass Silver Stain Solution (PSS-0125), which contains: Silver Stain [10X] 125ml Developer (dry powder mix) 75 g Sensitizer-I 4 ml Sensitizer-II 4 ml

Items Needed and Not Supplied:
Ethanol, glacial acetic acid and de-ionized water
Protocol Application Notes: Gel clarity will depend greatly on the quality of the reagents used in making and running the gel as well as the quality of protein samples loaded on the gel. Always use clean containers and highly purified de-ionized water for fixing, staining and washing the gel. Never touch the gel with fingers.
Reconstitute of the control proteins: Before opening the tube, centrifuge the tube at 15,000xg for 5 minutes. Carefully remove the storage buffer without disturbing the pellet if any. Air-dry the pellet for 5 minutes at room temperature. Suspend the proteins in 250µl SDS loading buffer. Use 5 or 10µl for each lane. Store the reconstituted protein control at -20°C.
Preparation before use:
Prepare the following reagent solutions:
1 Glyco-Fixing Solution: dilute fixing solution 5 fold in de-ionized water (e.g., dilute 10 ml of 5X solution in 40 ml deionized water for each mini gel)
2 Washing Solution I: 5 % acetic acid in de-ionized water (Prepare ~ 300ml /mini-gel)
3 Washing Solution II: 10 % ethanol, 5 % acetic acid in de-ionized water (Prepare ~ 300ml /mini-gel)
4 Glyco-Oxidizing Solution: weigh ~ 0.5g Glyco-Oxidizing Reagent and dissolve in 50ml de-ionized water for each minigel.
5 Glyco-Reducing Solution: weigh ~0.25g Glyco-Reducing Reagent and dissolve in 50ml de-ionized water for each minigel.
6 GlycoBlue Solution: dilute GlycoBlue solution 50 fold in Washing Solution II (e.g., dilute 1 ml GlycoBlue Solution in 50ml Washing Solution II).

Pre-warm Prepared Solutions and de-ionized water at 30 -35°C.
All the following staining steps should be performed with gently rocking.
Both GlycoBlue Staining Steps and Silver Staining Steps can be performed at room temperature.
Using pre-warmed solutions for GlycoBlue Staining Steps results in better resolution.

GlycoBlue Staining Steps:
1 After electrophoresis, transfer the gel to Glyco-Fixing Solution. Incubate for 15 min.
2 Wash the gel with Washing Solution I twice for 2 minutes each. Add the Glyco-Oxidizing Solution and incubate for 20 minutes.

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3 Wash the gel with Washing Solution I twice for 2 minutes each. Then wash the gel with de-ionized water twice for 2 minutes each. Add the Glyco-Reducing Solution and incubate for 10 minutes.
4 Wash the gel with de-ionized water twice for 2 minutes each, followed by washing with Washing Solution II twice for 2 minutes each.
5 Add the GlycoBlue Solution and Stain the gel for 20 minutes
6 Wash the gel three times in Washing Solution II for 1, 4 and 5 minutes.
7 Wash three times in de-ionized water for 5-10 minutes each at room temperature.

Silver Staining Steps:

1. **Silver stain preparation**: Dilute Silver Stain 10 fold (e.g., dilute 5 ml of the stain in 45ml de-ionized water) and then add 65 :l of Sensitizer-I. Soak the gel in diluted Silver Stain for 20-30 min while gently rocking.
   Developer preparation: While the gel is staining, prepare the developer. Add one heaping spoonful (~3gm) of Developer to 100 ml de-ionized water. After the developer is dissolved, add 65 µl Sensitizer-I and 65 µl Sensitizer-II.

1 Rinse the gel 10-20 seconds with de-ionized water. Soak the gel in Developer-Sensitizer-I & II. Gently rock the gel until bands are visible. Band intensity will develop quickly.
2 As soon as the band intensity reaches an acceptable level, stop the development with 2-5% acetic acid. Gel may be stored in 2-5 % acetic acid or water.

Differentiation between glycosylated and non-glycosylated proteins: Glycoprotein Staining kit stains both glycosylated and non-glycosylated proteins. Glycosylated proteins can be differentiated from the non-glycosylated proteins by excluding the glycosylated proteins dye staining steps. Run a control gel and develop the gel with only silver staining steps under identical conditions, in which glycosylated proteins will not stain (or stain faintly in some cases).
IMPORTANT-for best results, silver staining of the control gel should be performed together with the test gels and preferably in the same staining tray. Stop the gel development at the identical time interval.

Storage Condition:
The kits are shipped at ambient temperature. Upon arrival, store them at room temperature.