GLYCOPROTEIN DETECTION KIT

Product Information

Product Code **GLYCO-PRO**
Storage Temperature 0-5 °C

TECHNICAL BULLETIN

Product Description

Glycoprotein Detection Kit provides a system to easily detect the sugar moieties of glycoproteins on SDS-PAGE or on Western Blotting membranes. This detection system is a modification of Periodic acid-Schiff (PAS) methods[1,2] and yields magenta bands with a light pink or colorless background. The detection limits have been found to be 25-100 ng of carbohydrates depending on the nature and the degree of glycosylation of proteins. Peroxidase from horseradish, reported as having a carbohydrate content of approximately 16%,[3] is used as a positive control in the kit.

Reagents

Sufficient to stain 10 mini gels (8 x 10 cm) or 5 large gels (16 x 18 cm) or same sizes of blotting membranes.

Items Provided

Oxidation Component (Periodic Acid)	O 0258
Reduction Component (Sodium Metabisulfite)	R 0764
Schiff's Reagent, Fuchsin-Sulfite Reagent	S 5133
Peroxidase from Horseradish	P 2075

Items Required but not Provided

Methanol, Spectrophotometric Grade	M 3641
Acetic Acid, Glacial	A 6283
Water, 18 megohm cm resistivity	W 4502

Preparation Instructions

Technical Tips

1. Prepare all stock solutions using ultra pure water.
2. Wear gloves during each step.
3. Completely submerge the gel or membrane in each solution.
4. Perform staining procedure in a well-ventilated area or hood to remove aldehyde vapor generated during the oxidation step.
5. If unable to complete the staining procedure, leave gel(s) in the Fixing Solution overnight.

Preparation of Stock Solutions

1. Oxidation Component
   Add 950 ml of ultra pure water to the bottle labeled "Oxidation Component". Stir for approximately 15 minutes or until material is completely dissolved. Remove stirring bar, QS to 1000 ml and invert bottle several times to obtain a homogeneous solution. Store the solution at room temperature.
2. Reduction Component
   Add 950 ml of ultra pure water to the bottle labeled "Reduction Component". Stir for approximately 15 minutes or until material is completely dissolved. Remove stirring bar, QS to 1000 ml and invert bottle several times to obtain a homogeneous solution. Store the solution at room temperature.
3. Schiff's Reagent, Fuchsin-Sulfite Reagent
   No adjustment or dilution is necessary. Store at 0-5 °C.
4. Fixing Solution
   Prepare solution by combining 200 ml of water with 200 ml methanol (Product No. M 3641). Store at room temperature.
5. Storage Solution
   Combine 380 ml of ultra pure water with 20 ml of Acetic Acid, Glacial (Product No. A 6283). Store at room temperature.
6. Peroxidase Positive Control
   Reconstitute contents of vial with 0.5 ml of ultra pure water to produce a 2 mg/ml solution. Dilute to a 1 mg/ml solution with the sample buffer appropriate for the system being used. For a large gel (15 x 18 cm) load 10 µl of reconstituted positive control per lane and 5 µl per lane for mini gels (8 x 10 cm). After reconstitution of the Peroxidase Positive Control, aliquot and store at –20 °C.

Procedure

Use Table (A) to determine the time required for each step based on gel or membrane size and gel thickness.

Note: For staining a membrane, proceed from step 3 through step 7.

Step 1: Fixing
After electrophoresis, fix gel(s) by completely immersing in Fixing Solution. Gently agitate.

Step 2: Washing
Replace the Fixing Solution with ultra pure water and agitate gently. Repeat this step once.
Step 3: Oxidation
Transfer gel(s) or membrane(s) to Oxidation Solution and agitate gently.
Step 4: Washing
Replace the Oxidation Solution with ultra pure water and agitate gently. Repeat this step once.
Step 5: Staining
Replace the water with Staining Solution and agitate gently.
Step 6: Reduction
Replace Staining Solution with Reduction Solution and agitate gently.
Step 7: Washing
Replace the Reduction Solution with ultra pure water. Repeat 2-3 times. Note: The magenta band(s) will intensify during this step.
Step 8: Storage
Transfer gel(s) into 5% Acetic Acid Solution for storage.

References

1. Jay, G. D., et al., Anal. Biochem., **185**, 324 (1990).
2. Zacharius, R. M., et al., Anal. Biochem., **30**, 148 (1969).
3. Racusen, D., Anal. Biochem., **99**, 474, (1979).

Table (A): Recommended Gel or Membrane Staining Conditions

Steps	Volumes		Time for gel thickness 0.5-0.75 mm or for membrane	Time for gel thickness 1.0-1.5 mm
	Size e 16 x 18 cm	Size 8 x 10 cm		
1. Fixing	400 ml	200 ml	30 min.	60 min.
2. Washing	400 ml	200 ml	2 x 10 min.	2 x 20 min.
3. Oxidation	200 ml	100 ml	30 min.	60 min.
4. Washing	400 ml	200 ml	2 x 10 min.	2 x 20 min.
5. Staining	200 ml	100 ml	1-2 hours or until bands turn magenta	1-2 hours or until bands turn magenta
6. Reduction	200 ml	100 ml	60 min.	120 min.
7. Washing	400 ml	200 ml	Band color will intensify with changes of fresh water	Band color will intensify with changes of fresh water
8. Storage	400 ml	400 ml	overnight	overnight

MAM/KMR 05/02