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Product Information

Glycoprotein Detection Kit

Catalog Number **GLYCOPRO**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Glycoprotein Detection Kit provides a system to easily detect the sugar moieties of glycoproteins on SDS-PAGE gels 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 limit has been found to be in the range of 25-100 ng for carbohydrates depending on the nature and the degree of glycosylation of the protein. Horseradish peroxidase is reported to have a carbohydrate content of approximately 16%[3] and is used as a positive control in the kit.

Components

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

Oxidation Component (Periodic Acid)	O0258
Reduction Component (Sodium Metabisulfite)	R0764
Schiff's Reagent, Fuchsin-Sulfite Reagent	S5133
Peroxidase from Horseradish	P2075

Reagents Required but Not Provided

Methanol	494437
Acetic Acid, Glacial	A6283
Ultrapure Water (18 MΩ•cm or equivalent)	

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Prepare all stock solutions using ultrapure water.

- Oxidation Solution - Add 950 ml of ultrapure water to the bottle labeled Oxidation Component (Catalog Number O0258). Stir for ~15 minutes or until the material is completely dissolved. Remove the stirring bar and bring the final volume to 1,000 ml. Invert the bottle several times to obtain a homogeneous solution. Store the Oxidation Solution at room temperature.
- Reduction Solution - Add 950 ml of ultrapure water to the bottle labeled Reduction Component (Catalog Number R0764). Stir for ~15 minutes or until the material is completely dissolved. Remove the stirring bar and bring the final volume to 1,000 ml. Invert the bottle several times to obtain a homogeneous solution. Store the Reduction Solution at room temperature.
- Schiff's Reagent, Fuchsin-Sulfite Reagent (Catalog Number S5133) – The reagent is supplied ready-to-use and no adjustment or dilution is necessary. Store the reagent at 2–8 °C.
- Fixing Solution - Prepare the Fixing Solution by combining 200 ml of water with 200 ml of methanol (Catalog Number 494437). Store the Fixing Solution at room temperature.
- Storage Solution - Combine 380 ml of ultrapure water with 20 ml of glacial acetic acid (Catalog Number A6283). Store the Storage Solution at room temperature.
- Peroxidase Positive Control - Reconstitute the contents of the vial with 0.5 ml of ultrapure water to produce a 2 mg/ml solution. Dilute this solution to 1 mg/ml with the sample buffer appropriate for the system being used. Boil an appropriate volume of the prepared control for 1-3 minutes prior to loading on the gel. For a large gel (15 x 18 cm), load 10 µl of the 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 1 to determine the time required for each step based on gel or membrane size and gel thickness. If unable to complete the entire procedure in one day, it is acceptable to store gels for an extended period (overnight) at several points. Gels can be stored overnight following fixing (step 1) or following staining (step 5). Gels stored after fixing can be stored in fixing solution or water. Gels stored following staining should be stored in staining solution.

Perform this staining procedure in a well-ventilated area or hood to remove aldehyde vapor generated during the oxidation step. It is recommended to wear gloves during each step.

Best results are obtained by completely submerging the gel or membrane in each solution.

Note: For membrane staining, start at step 3 and proceed through step 7.

1. Fixing
   After electrophoresis, fix the gel(s) by completely immersing in the Fixing Solution. Gently agitate.
2. Washing
   Replace the Fixing Solution with ultrapure water and agitate gently. Repeat this step once.
3. Oxidation
   Transfer the gel(s) or membrane(s) to the prepared Oxidation Solution and agitate gently.
4. Washing
   Replace the Oxidation Solution with ultrapure water and agitate gently. Repeat this step once.
5. Staining
   Replace the water with Schiff's Reagent and agitate gently.
6. Reduction
   Replace the Schiff's Reagent with the Reduction Solution and agitate gently.
7. Washing
   Replace the Reduction Solution with ultrapure water. Repeat 2-3 times.
   Note: The magenta band(s) will intensify during this step.
8. Storage
   Transfer the gel(s) into the Storage Solution (5% acetic acid solution).

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).

GL,MAM,KMR 10/16-1

Table 1.
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 16 x 18 cm	Size 8 x 10 cm		
1. Fixing	400 ml	200 ml	30 minutes	60 minutes
2. Washing	400 ml	200 ml	2 × 10 minutes	2 × 20 minutes
3. Oxidation	200 ml	100 ml	30 minutes	60 minutes
4. Washing	400 ml	200 ml	2 × 10 minutes	2 × 20 minutes
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 minutes	120 minutes
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