



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, Spectrophotometric Grade | M3641 |
| 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 Material 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 M3641). 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).

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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 |

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