

Glycoprotein Western Detection Kit Protocol-at-a-Glance

(PT4056-2)

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The **Glycoprotein Western Detection Kit** (Cat. No. 635648) is designed for detection of glycoprotein sugar moieties on Western blotting membranes. The kit contains sufficient materials to stain 20 Western blots. The blots may be generated by performing gel electrophoresis and Western blotting to nitrocellulose or PVDF membranes using your standard laboratory methods, according to the instructions provided by the respective manufacturer of your reagents.

A. Kit Components

- Oxidizing Reagent (3 x 85 ml)
- Staining Reagent (3 x 85 ml)
- HRP Positive Control (250 µl; apply 5–10 µl per lane)

B. Additional Reagents Required

- 3% acetic acid

Mix 15 ml of glacial acetic acid with 485 ml of ultrapure water. Store at room temperature.

C. Detection Procedure

Perform this procedure in a fume hood, since the Staining Reagent is toxic.

1. Wash membrane with 20 ml of 3% acetic acid for 5 min on an orbital shaker.
Repeat this step once.
2. Transfer membrane to a container holding 10 ml of Oxidizing Reagent and gently agitate on an orbital shaker for 20 min.
3. Wash membrane with 10 ml of 3% acetic acid for 5 min on an orbital shaker.
Repeat this step twice.
4. Transfer membrane to a container holding 10 ml of Staining Reagent and gently agitate on an orbital shaker for 15 min.
5. Wash membrane extensively with 3% acetic acid and then with ultrapure water.
Glycoproteins will appear as magenta bands.

NOTE: Oxidizing Reagent may be stored at room temperature. If a precipitate is present after warming to room temperature, then spin briefly in a centrifuge and use supernatant for detection.

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