Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Glycoprotein Staining Kit

01/21

(Catalog# K2073-20; Store at 4 °C)

I. Introduction:

BioVision's Glycoprotein Staining Kit is designed for the staining and detection of glycoproteins on a PVDF or nitrocellulose membrane using the periodic acid-Schiff (PAS) method. The kit provides all the necessary reagents needed to specifically stain the glycoproteins separated by polyacrylamide gel electrophoresis and transferred to a nitrocellulose or PVDF membrane. The glycoproteins are detected as magenta bands against a colorless background. The kit contains sufficient reagents for staining up to 20 membranes (6 x 9 cm) and can detect as low as 0.5 µg of native glycoproteins.

II. Application:

- Detection of glycoproteins on a PVDF or Nitrocellulose membrane.

III. Kit Contents:

Components	K2073-20	Cap Code	Part Number
Oxidizing Reagent (10X)	20 ml	NM	K2073-20-1
Staining Reagent A (6X)	40 ml	NM	K2073-20-2
Staining Reagent B (6X)	40 ml	NM/Green	K2073-20-3
Glycoprotein Positive Control	1 vial	Red	K2073-20-4
Glycoprotein Negative Control	1 vial	Blue	K2073-20-5

IV. User Supplied Reagents and Equipment:

- Equipment's to run SDS-PAGE and transfer proteins from the gel to a PVDF or nitrocellulose membrane.
- 3% glacial acetic acid (i.e 15 ml of glacial acetic acid in 485 ml of water). Store at room temperature (RT).

V. Storage Conditions and Reagent Preparation:

Store the kit at 4 °C, protected from light. Mix well before opening. Read the entire protocol before performing the assay.

- **Oxidizing Reagent Working Solution:** Prepare the Oxidizing Reagent Working Solution by mixing one part of Oxidizing Reagent (10X) with nine parts of 3% glacial acetic acid and mix well. Store at 4 °C.
- **Staining Reagent Working Solution:** Prepare fresh Staining Reagent Working Solution by mixing one part of Staining Reagent A (6X), one part of Staining Reagent B (6X) with four parts of water.
- **Glycoprotein Positive & Negative Controls:** Reconstitute each vial in 250 µl of water to make 1 mg/ml solution. Divide into aliquots and store at -20 °C.

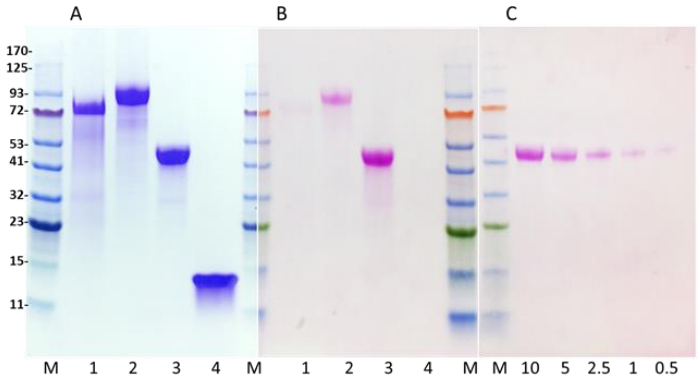
VI. Glycoprotein Staining Protocol:

1. SDS-PAGE: Run a SDS-PAGE to separate the glycoproteins, following the standard SDS-PAGE procedure.

2. Gel Transfer: Transfer the proteins from the gel onto a PVDF or Nitrocellulose membrane using standard gel transfer procedure.

3. Staining of Glycoproteins on membrane:

a. Wash the membrane with 20 ml of 3% glacial acetic acid for 5 min with gentle shaking. Repeat this step once.
b. Add 10 ml of **Oxidizing Reagent Working Solution** and gently shake for 20 min.
c. Wash the membrane with 10 ml of 3% glacial acetic acid for 5 min with gentle shaking. Repeat this step twice.
d. Add 12 ml of **fresh Staining Reagent Working Solution** to the container with the membrane and gently shake for 15 min.
e. Wash the membrane briefly with 10 ml of 3% glacial acetic acid and water.
f. Take the image immediately. **Glycoproteins will appear as magenta bands. Note:** The back ground will become darker over time.



Figures A. SDS-PAGE and Coomassie staining of various proteins. B. Selective staining of glycoproteins. Lanes, M: Protein Marker; 1: Deglycosylated recombinant glycoprotein; 2: Recombinant glycoprotein; 3: Positive control (native glycoprotein); 4: Negative control. **C. Glycoprotein staining using various amounts of native glycoprotein** (10, 5, 2.5, 1 and 0.5 µg respectively). For deglycosylation, 10 µg of glycoprotein (BioVision Cat# P1172) was treated with 0.1 mU of PNGase F (BioVision Cat# P1598) for 2 hr at 37 °C.

VII. Related Products:

- Sialic Acid (NANA) Colorimetric/Fluorometric Assay Kit (Cat# K566)
- Alpha-Mannosidase Activity Assay Kit (Fluorometric) (Cat# K2041)
- Beta-Mannosidase Activity Assay Kit (Fluorometric) (Cat# K2045)
- Sialyltransferase Activity Assay Kit (Fluorometric) (Cat# K2048)
- PNGase F, Recombinant (Cat# P1598)
- PNGase F Deglycosylation Kit (K2061)

FOR RESEARCH USE ONLY! Not to be used on humans.