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

PNGase F Deglycosylation Kit

11/20

(Catalog #K2061-100; Store at -20 °C)

I. Introduction:

Peptide-N4-(N-acetyl-β-D-glucosiminyl asparagine amidase F (PNGase F) is an amidohydrolase that cleaves the N-glycan chains at the innermost *N*-Acetylglucosamine (GlcNAc) of glycans and asparagine residues of high mannose, hybrid and complex oligosaccharides of N-linked glycoproteins and glycopeptides. The cleavage results in a deaminated protein or peptide and free glycans. PNGase F is widely used in the deglycosylation of glycoproteins to study glycans and identify glycosylation sites. **BioVision's PNGase F Deglycosylation Kit** is designed for the quick deglycosylation of glycoproteins using PNGase F under optimized conditions. The kit provides all the necessary reagents for the deglycosylation at least 20 mg of glycoproteins.

II. Applications:

- Removal of glycans from N-linked glycopeptides and glycoproteins.
- Structural and functional study of N-linked glycoproteins.
- Preparation of glycans and proteins of glycoprotein for mass analysis, crystallization and structural studies.

III. Kit Contents:

Components	K2061-100	Cap Code	Part Number
PNGase F Reaction Buffer (5X)	1 ml	White	K2061-100-1
Denaturation Buffer (10X)	0.5 ml	Blue	K2061-100-2
Detergent Solution (10X)	0.5 ml	Orange	K2061-100-3
Recombinant PNGase F	200 µl	Green	K2061-100-4
Glycoprotein Control	1 vial	Yellow	K2061-100-5

IV. User Supplied Reagents and Equipment:

- Equipment to run SDS-PAGE
- Staining & destaining reagents

V. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **PNGase F Reaction Buffer (5X), Denaturation Buffer (10X) & Detergent Solution (10X):** Warm to room temperature (RT) before use. Store at -20 °C.
- **Recombinant PNGase F:** Ready to use. Divide into aliquots and store at -20 °C. Keep on ice, while in use. Avoid freeze and thaw cycles. Use within two months.
- **Glycoprotein Control:** Reconstitute the vial in 40 µl of distilled water to prepare Glycoprotein Control solution. Divide into aliquots & store at -20 °C.

VI. Deglycosylation Assay Protocol:

1. Preparation of Glycoprotein Sample: Prepare **Glycoprotein Sample** in 1X PNGase F Reaction Buffer.

2. Preparation of Deglycosylation Mix: For each Glycoprotein Sample, prepare 45 µl of Deglycosylation Mix containing:

	Deglycosylation Mix
PNGase F Reaction Buffer (5X)	10 µl
Denaturation Buffer (10X)	5 µl
Glycoprotein Sample	50-500 µg (in 30 µl)
Adjust the volume to (with water)	45 µl

3. Mix well and heat at 100 °C for 10 min.
4. Spin briefly and cool down the solution.
5. Add 5 µl of 10X Detergent Solution and mix well.
6. Add 2 µl of Recombinant PNGase F to the above solution. Mix well and Incubate at 37 °C for 2 hr.
7. Stop the enzymatic reaction by putting the vial on ice. This is the **deglycosylated sample**.

Notes:

a. The amount of Recombinant PNGase F needed for the complete deglycosylation might vary depending on the Glycoprotein Sample(s). We recommend performing a pilot experiment to determine the ratio of glycoprotein sample to PNGase F and the incubation time.
b. Dilute the Recombinant PNGase F in 1X PNGase F Reaction Buffer, if required.
8. Analyze the deglycosylated sample on a gradient SDS-PAGE, while keeping the remaining reaction mix on ice. The remaining reaction mix can be lyophilized or frozen for downstream applications including mass spectrophotometric analysis of the deglycosylated protein etc.

Glycoprotein Control (optional): As a Positive Control, use 10 µl of Glycoprotein Control solution instead of Glycoprotein Sample in the above Deglycosylation Mix and follow step 2 to step 6, but reduce the volume of the reagents accordingly.

VII. SDS-PAGE Analysis of Deglycosylated Sample(s): Add 5 µl of 3X SDS-loading buffer (BioVision Cat# 2108, not supplied) to 10 µl of deglycosylated sample(s) (from step 7) and heat for 5 min at 100 °C. Cool down the sample(s) and load 5-10 µl on a gradient SDS-PAGE. Load equal amount of non-deglycosylated glycoprotein sample as the Negative Control.

VIII. Results: Several native glycoproteins and recombinant proteins were tested as shown below**.**

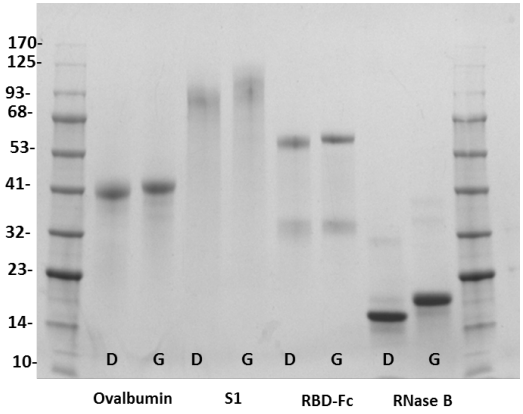

Figure A. 20 µg of various glycoproteins were deglycosylated using 0.1 mU of PNGase F (BioVision Cat# P1598) for 1 hr at 37 °C. The deglycosylation was monitored on a 4-20% gradient SDS-PAGE. 3 µg of Ovalbumin; Recombinant Sars-Cov-2 Spike protein 1 (BV Cat# P1653); Recombinant Sars-Cov-2 Spike protein 1 receptor-binding domain (RBD-Fc) (BV Cat# P1654) and RNase B were used for the deglycosylation studies. **D:** Deglycosylated glycoprotein. **G:** Glycoprotein.

IX. 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)

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