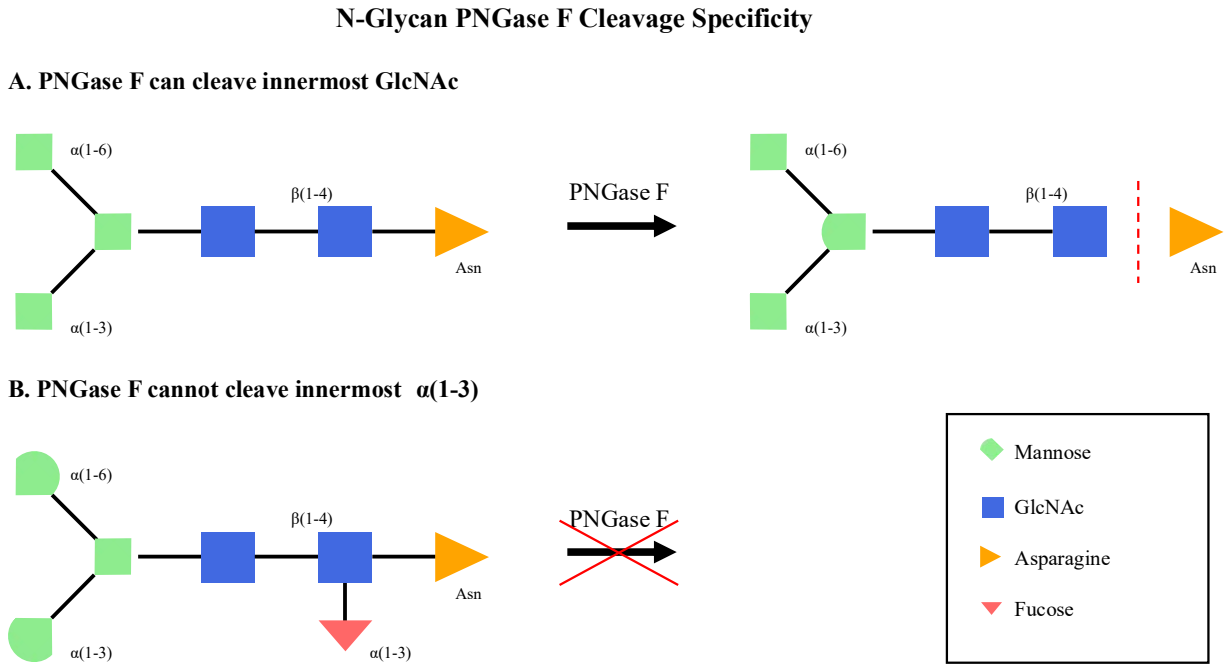
## Description

The PNGase F Deglycosylation Kit is designed for the simple, *in vitro* deglycosylation of glycoproteins. All the necessary buffers are included to ensure >95% deglycosylation of glycoproteins under denaturing or non-denaturing conditions. Additionally, a positive control is included for experimental optimization and validation. This kit comes with enough reagents for 60 deglycosylation reactions of 20 µl with up to 50 µg of protein (up to 3mg of protein in total).

## Background

PNGase F (Peptide:N-glycosidase F) is an amidase that catalyzes the hydrolysis of the amide bond between the innermost N-acetylglucosamine (GlcNAc) and asparagine residues of high mannose, hybrid, and complex oligosaccharides, resulting in a deaminated protein/peptide and a free glycan. One notable exception to its activity is glycans containing α(1,3)-linked core fucose residues, commonly found in plant glycoproteins, which remain resistant to PNGase F cleavage. PNGase F is a cornerstone tool in glycoproteomics, enabling researchers to examine protein structure and function without interference from N-linked glycans. This capability has proven essential for




applications ranging from protein characterization to biomarker discovery.

*Figure 1. PNGase mode of action.*

PNGase F cleaves innermost GlcNAc. It cannot cleave when the GLcNAc is linked to an a1-3 Fucose residue, a modification commonly found in plants and insect glycoproteins.

**Applications**

- Specific removal of N-linked glycans from proteins for glycosylation site identification, protein structural analysis, and glycan characterization.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 101753-KC60 | PNGase F (500 Units/µl) | 60 µl | -20°C |
| 82881-KC1 | 10x Reaction Buffer | 1 ml | -20°C |
| 82882-KC1 | 10x Denaturing Buffer | 1 ml | -20°C |
| 82883-KC1 | 10% NP-40 | 1 ml | -20°C |
| 82884-KC10 | RNase B Positive Control (5 mg/ml) | 10 µl | -20°C |

**Unit Definition**

One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 20 µl.

**Materials Required but Not Supplied**

- Glycoprotein of interest
- Heat block able to reach 100°C
- Water bath/incubator able to reach 37°C
- Microfuge

**Stability**

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

**Safety**

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

**Contraindications**

- Do not use SDS (sodium dodecyl sulfate). To ensure optimal PNGase F activity, it is crucial to include NP-40 in the reaction mixture due to the inhibitory effects of SDS.

**PNGase F Protocol**

- Deglycosylation of native proteins may require further optimization (e.g., longer incubation, more enzyme, etc.).
- This is a general guideline only, optimization may be required for the specific target of interest.
- The use of the Positive Control provided is recommended.

**A. Reaction using denaturing conditions**

1. Thaw all reagents on ice.
2. Mix well by gently flicking the tube and/or pipetting up and down.
3. Do a quick spin in a microfuge.
4. In a new Eppendorf tube, add up to 50 µg of the target glycoprotein. The volume should be 13 µl (add distilled water if necessary to reach the recommended volume).
5. Prepare a Positive Control reaction tube with 2 µl of Positive Control and 11 µl of distilled water.
6. Add 2 µl of 10x Denaturing Buffer and mix gently.
7. Denature the sample by heating at 100°C for 10 minutes.
8. Cool to Room Temperature (RT) prior to adding the enzyme.
9. Add 2 µl of 10x Reaction Buffer to all tubes.
10. Add 2 µl of 10% NP-40 to all tubes.
11. Add 1 µl of PNGase F to all tubes and mix gently.
12. Incubate at 37°C for 1 hour.
13. Analyze by performing mobility shift assays on SDS-PAGE gels.

**B. Reaction using non-denaturing conditions**

- If using non-denaturing conditions, it is recommended that an additional reaction using denaturing conditions is performed to provide a >95% deglycosylated control.
- The use of the Positive Control provided is recommended.

1. Thaw all reagents on ice.
2. Mix well by gently flicking the tube and/or pipetting up and down.

3. Do a quick spin in a microfuge.

4. In a new Eppendorf tube, add up to 50 µg of the target glycoprotein. The volume should be 17 µl (add distilled water if necessary to make the recommended volume).

5. Prepare a Positive Control reaction tube with 2 µl of Positive Control and 15 µl of distilled water.

6. Add 2 µl of 10x Reaction Buffer to all tubes.

7. Add 1 µl of PNGase F to each tube and mix gently.

8. Incubate at 37°C for 4-24 hours.

9. Analyze by performing mobility shift assays on SDS-PAGE gels.

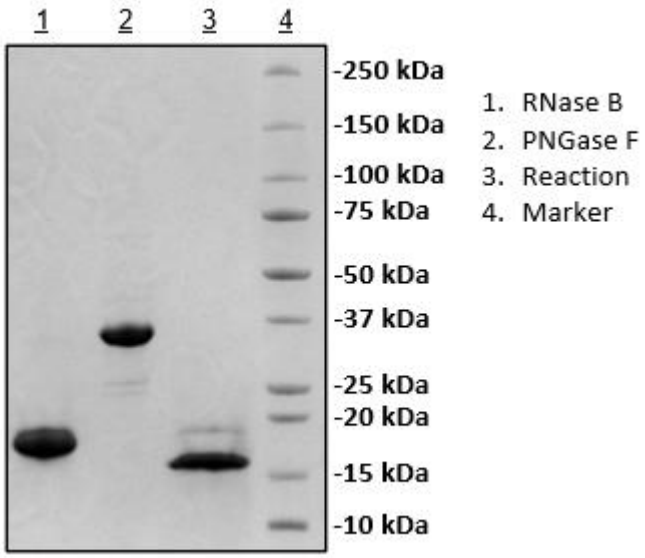



*Figure 2. Activity assessment of PNGase F by SDS-PAGE.*
1U of PNGase F was incubated with 10 µg of denatured RNase B for 1 hour at 37°C in a total volume of 20 µl. This resulted in >95% removal of carbohydrates. Deglycosylation is observed as a mobility shift of RNase B from ~18 kDa to ~16 kDa by SDS-PAGE. Lane 1: RNase B (10 µg); Lane 2: PNGase F (4 µg); Lane 3: RNase B (10 µg) + PNGase F (1 U).

*Data shown is representative.*

**Troubleshooting Guide**
For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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