PNGase F Fly

Please read the manual carefully before use.

Cat. No. LG102

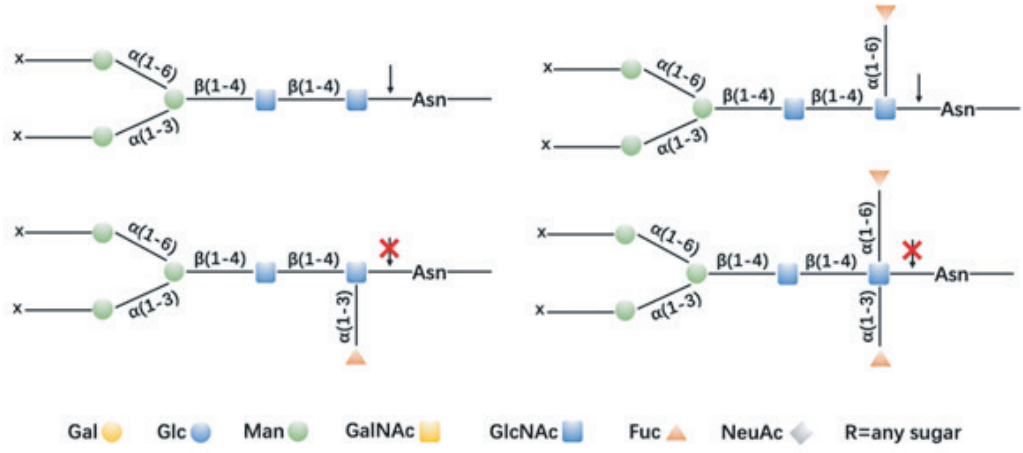
Version No. Version 1.0

Storage: At -18℃ or below for two years.

Description

Peptide N-Glycosidase F (PNGase F) is an amidase derived from *Elizabethkingia miricola*, genetically engineered for expression in *Escherichia coli*. This enzyme is the most effective enzymatic method for removing nearly all N-linked glycans from glycoproteins and plays a critical role in glycoprotein research. PNGase F cleaves N-linked glycans from glycoproteins, including high-mannose, hybrid, and complex-type oligosaccharides. The cleavage site is the glycosidic bond between the innermost N-acetylglucosamine (GlcNAc) of the glycan chain and the asparagine residue. In antibody glycosylation studies, obtaining accurate N-glycan profiles as quickly as possible is essential. Using the standard version of PNGase F, the release of N-glycans from antibodies typically requires several hours of incubation, followed by liquid chromatography or mass spectrometry analysis. PNGase F Fly is an improved reagent that enables rapid deglycosylation of antibodies within minutes. All N-glycans are released quickly and without bias, allowing for rapid glycoprofiling of glycosylated antibodies.

Mechanism of Action of PNGase F



PNGase F cleaves N-linked glycans from glycoproteins if the innermost GlcNAc residue is linked to an α1-6 fucose residue. However, PNGase F cannot cleave N-linked glycans when the innermost GlcNAc residue is linked to an α1-3 fucose residue — a modification commonly found in plant and some insect glycoproteins.

Kit Contents

Component	LG102-01 (50 rxns)
PNGase F Fly	50 µl
5×PNGase F Fly Buffer	200 µl

Enzyme Storage Buffer

20 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, 50% glycerol, pH 7.5 @ 25°C

Protocol

I. One-Step Procedure

1. Dissolve 100 µg of antibody or glycoprotein in deionized water, and add deionized water to bring the volume to 16 µl.
2. Add 4 µl of 5× PNGase F Fly Buffer to reach a total volume of 20 µl. Mix gently by pipetting.

3. Add 1 µl of PNGase F Fly and mix gently by pipetting.
4. Incubate at 50°C for 10 minutes.
5. The sample can then be analyzed by routine methods such as SDS-PAGE. Alternatively, N-glycans can be derivatized (e.g., by reductive amination) for downstream analysis. If the sample is intended for mass spectrometry analysis, buffer exchange by dialysis or ultrafiltration is required.

II. Two-Step Procedure

Some antibodies (e.g., those with Fab N-glycans) require a pre-heating step for efficient deglycosylation.

1. Dissolve 100 µg of antibody or glycoprotein in deionized water, and add deionized water to bring the volume to 16 µl.
2. Add 4 µl of 5× PNGase F Fly Buffer to reach a total volume of 20 µl. Mix gently by pipetting.
3. Incubate at 80°C for 2 minutes, then cool on ice.
4. Add 1 µl of PNGase F Fly and mix gently by pipetting.
5. Incubate at 50°C for 10 minutes.
6. The sample can then be analyzed by routine methods such as SDS-PAGE. Alternatively, N-glycans can be derivatized (e.g., by reductive amination) for downstream analysis. If the sample is intended for mass spectrometry analysis, buffer exchange by dialysis or ultrafiltration is required.

Notes

- Ensure that the PNGase F Fly Buffer is completely thawed and thoroughly mixed before use.
- Before incubation, ensure that the PNGase F Fly Buffer, the antibody or glycoprotein sample, and PNGase F Fly are mixed uniformly.
- The reaction system can be scaled proportionally. Adjust the amount of PNGase F Fly accordingly. Changing component ratios without proportional scaling (e.g., using 1 µl of enzyme in a larger reaction volume) may compromise digestion efficiency.
- Incubation at 50°C for 3-5 minutes is usually enough. Longer incubation (>15 minutes) does not improve results. If deglycosylation is incomplete, try the two-step procedure (with 80°C pre-denaturation).

Applications

1. Proteomics Research
   Study the effects of glycosylation on protein function, stability, or protein-protein interactions.
   Remove glycans prior to mass spectrometry analysis to simplify protein structures and improve identification accuracy.
2. Biopharmaceuticals (e.g., Antibody Drugs)
   Analyze glycan profiles of monoclonal antibodies (e.g., IgG) to evaluate the impact of glycosylation on drug efficacy.
   Detect glycosylation heterogeneity during quality control.
3. Glycoengineering and Recombinant Protein Production
   Validate glycosylation sites or prepare aglycosylated protein controls.
4. Disease Research
   Analyze aberrant glycosylation biomarkers in diseases such as cancer and immune disorders.

FAQ & Solutions

1. What is the difference between PNGase F Fly and PNGase F?
   PNGase F Fly enables deglycosylation of antibodies and glycoproteins within minutes. Its convenient reaction workflow and rapid reaction time make it suitable for high-throughput applications in the development and quality control of therapeutic monoclonal antibodies.
2. How to choose between the one-step and two-step procedures?
   PNGase F Fly enables efficient and rapid deglycosylation of antibodies using the one-step procedure at 50°C. However, some IgG antibodies (e.g., those carrying Fab glycans) require pre-denaturation at 80°C for 2 minutes. We recommend first trying the one-step procedure. If N-glycans remain (e.g., gel shift or proteomics evidence), use the two-step procedure.

3. How to determine if PNGase F Fly has completed the reaction within 10 minutes?
For monoclonal antibodies, removal of N-glycans can be observed as a band shift on SDS-PAGE. Typically, the band positions of the control sample and the treated sample should be compared on the same SDS-PAGE gel to determine partial or complete deglycosylation. If a more sensitive method is required to detect whether small amounts of glycosylated antibody remain, techniques such as LC-ESI-TOF mass spectrometry can be used, which resolve different glycosylated and non-glycosylated forms.

Item	Standard
Appearance	Colorless, clear
Molecular Weight	36 kDa
Isoelectric Point (pI)	7.64
Purity	≥95% (by SDS-PAGE)
Exoglycosidase	Not detected
Endoglycosidase F1	Not detected
Endoglycosidase F2	Not detected
Endoglycosidase F3	Not detected
Endoglycosidase H	Not detected
Protease	Not detected

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