

Active Recombinant PNGase F

Cat. No. PNGase F-15 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	PNGase F is recombinantly expressed in E.coli. Residual Host Cell Protein: <50 ppm
Source	E.coli
Description	It can selectively hydrolyze all N-glycan chains linked to Asn in proteins, except those containing α 1-3 fucosidic bonds. The cleavage site is the glycosidic bond between Asn and the innermost GlcNAc. This causes the glycoprotein to release the Asn-linked sugar molecules and converts Asn to Asp. The cleaved glycan types include high-mannose, hybrid, and complex oligosaccharides.
Form	Lyophilized
Purity	$\geq 95\%$ by SDS-PAGE
Advantages	1. High purity: It is free from contamination by other proteases and has no other endo- and exo-glycosidase activities, with a purity of $\geq 95\%$; 2. High stability: Each batch of PNGase F undergoes strict quality control to ensure batch-to-batch stability of the product; 3. High specific activity: It can effectively and completely release N-linked glycans; 4. Compatible with HPLC/ MS: The lyophilized form of PNGase F does not contain glycerol, which helps to achieve optimal results in HPLC and MS analyses; 5. This product can cleave glycoproteins under denaturing and non-denaturing conditions.
Protein Content	≥ 20 KU
Unit definition	One unit is defined as the amount of the enzyme required to remove more than 95%

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of the carbohydrates from 10 µg of denatured RNase B in a 10 µL reaction system at 37 centigrade for 1 hour.

Usage

1. Protein Reconstitution Centrifuge the centrifuge tube containing lyophilized PNGase F at 100-200 g for 2 minutes. Carefully open the cap and add pure water to achieve a final concentration of 500 U/µL. Invert the tube up and down to mix thoroughly as much as possible. After mixing, centrifuge at 100-200 g for 2 minutes. The reconstituted PNGase F should be stored at -20 centigrade for 6 months, and users should try to avoid repeated freeze-thaw cycles. 2. Denaturing enzymatic digestion Dissolve 1-20 µg of glycoprotein in deionized water. Add 1 µL of 10× glycoprotein denaturation buffer and adjust the volume to 10 µL with deionized water. Incubate at 100 centigrade for 10 min. Transfer to ice and centrifuge for 10 s. Add 2 µL of 10× PNGase F digestion reaction buffer, 2 µL of 10% NP-40, and 5 µL of deionized water. Mix well. Add 1 µL of the reconstituted PNGase F. Incubate at 37 centigrade for 60 min. Analyze the effect of enzymatic digestion. 3. Non-denaturing enzymatic digestion Dissolve 1-20 µg of glycoprotein in deionized water. Add 1 µL of 10× PNGase F digestion reaction buffer and 2-4 µL of the reconstituted PNGase F, then adjust the volume to 10 µL with deionized water. Incubate at 37 centigrade for 2 hours to overnight. Analyze the effect of enzymatic digestion.

Note

1. Denaturing enzymatic digestion has a relatively high efficiency and should be attempted first. If denaturing enzymatic digestion cannot be carried out (for example, precipitation occurs during the denaturation process), non-denaturing enzymatic digestion can be tried. However, the digestion efficiency may decrease. It is recommended to increase the amount of enzyme and extend the digestion time; 2. After reconstitution, try to avoid repeated freeze-thaw cycles for this product; 3. Please wear lab coat and disposable gloves when using; 4. This product should not be used directly for clinical diagnosis and treatment.

Storage

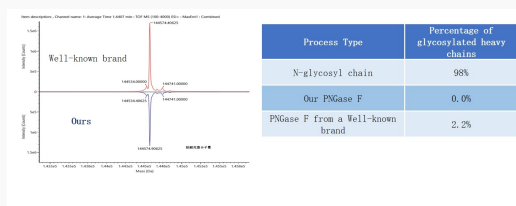
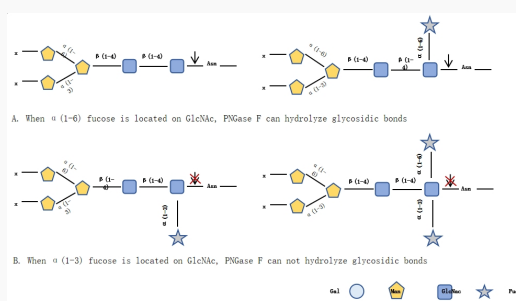
The PNGase F, Lyophilized should be stored at 2-8 centigrade. The 10× glycoprotein denaturation buffer, 10× PNGase F digestion reaction buffer and 10% NP-40 should be stored at -20 centigrade and can be preserved for at least 12 months.

Shipping

Dry ice or ice packs

Reconstitution

Ultrapure water



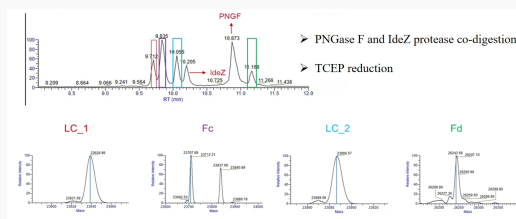
Deglycosylation

Design: After the samples are pretreated, they should be digested separately with our PNGase F and that from a well-known brand at 37 centigrade for 2h, and then supernatant was taken for testing. result: The complete molecular weight analysis of glycoproteins after deglycosylation showed that, the digestion effect of our PNGase F is consistent with that of the well-known brand.

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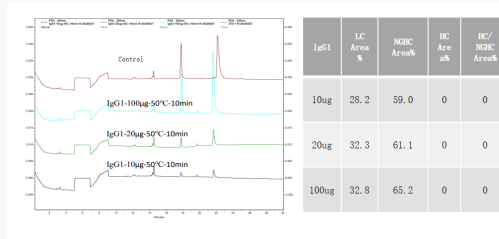
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Co-digestion

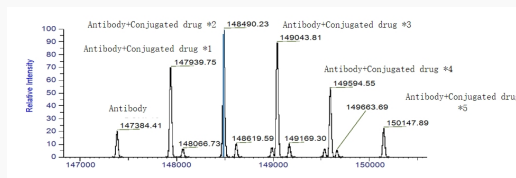
Design: Add PNGase F and IdeZ protease to the pretreated mixed antibody for enzymatic digestion. After reduction with TCEP, perform MS analysis. result: The efficiency of the combined enzymatic digestion is significantly improved, and correct antibody fragments are obtained.



Rapid digestion

Design: Under non-denaturing conditions, the monoclonal antibody was digested with PNGase F at 50 centigrade for 10min, and then analyzed by mass spectrometry. result: After digestion at 50 centigrade for 10 minutes, 500 U of PNGase F can completely cleave the N-glycans of the 10-100ug monoclonal antibody IgG1.

de-N-glycosylation analysis



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Design: RDC samples were digested with PNGase F under non-denaturing conditions for 2h. result: The N glycosylation sites at position N298 are all completely deglycosylated.

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