PNGase F Glycan Cleavage Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The PNGase F Glycan Cleavage Kit includes all components necessary to perform the enzymatic removal of almost all N-linked oligosaccharides from glycoproteins. The kit includes recombinant Peptide N-Glycosidase F (PNGase F) enzyme, which cleaves N-glycan chains at the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides, and a 10X reaction buffer.

Contents and storage

Content	Amount	Storage[1]
Recombinant, PNGase F (500,000 units[2])	66 µL	–20°C
PNGase F 10X Buffer	1 mL	

[1] Stable for up to 24 months.

[2] 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 10 µL.

General guidelines

- Do not use SDS in the reaction mixture because SDS inhibits PNGase F activity. If detergent is needed, use NP-40.
- N-linked glycans containing core α1-3 Fucose are not cleaved by PNGase F.
- For deglycosylation of native glycoprotiens, increased incubation time and increased amount of enzyme may be needed.
- Optimize reaction conditions for each substrate.
- If a larger amount of glycoprotein is used, scale up reaction volumes accordingly.

Digest glycoprotein with PNGase F (non-denaturing conditions)

Note: The following instructions are for one sample. The Master Mix volume can be scaled up proportionally for multiple samples.

1. Prepare a Master Mix by combining 4 µL of PNGase F 10X Buffer with 0.5 µL of PNGase F enzyme.
2. Add 25 µg of glycoprotein sample to a 1.5-mL tube, then bring the reaction volume up to 35.5 µL with HPLC-grade water.
3. Add 4.5 µL of the prepared Master Mix, then mix gently.
4. Incubate the reaction at 50°C for 1 hour, then centrifuge briefly.
5. Analyze by method of choice.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Revision	Date	Description
A.0	14 April 2019	New document.