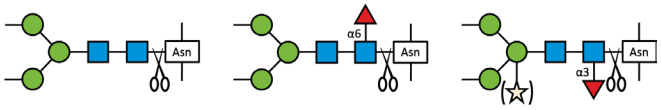
PNGase F-II contents

Catalog #	Description	Size	M. W.	Purity	pH	Storage
GE0201	PNGase F-II	100 units, lyophilized	62,300	> 95%	6.5-7.5 optimal	-20°C, up to 12 months
BA0601	10X Reaction Buffer 2	1 mL			7.0	4 to 25°C

This product is for research use only and not for resale or for any use in the manufacture of a therapeutic or for any diagnostic purpose.

Product description: This product is recombinant PNGase F-II, cloned from *Elizabethkingia meningosepticum* and expressed in *Escherichia coli* with an *N*-terminal 6xHis tag. It catalyzes the cleavage of asparagine-linked oligosaccharides, with or without core fucose, from glycoproteins and glycopeptides. ***Unlike PNGase F, which cannot cleave N-glycans with α1,3-linked core fucose, PNGase F-II can cleave N-glycans with α1,6- or α1,3-linked core fucose.***



This product does not contain any detectable activities of proteases or other glycosidases.

Unit definition: One unit is defined as the amount of PNGase F-II required to deglycosylate 1 nanomole (15 µg) of denatured RNase B or 0.1 nanomole (4.5 µg) of horse radish peroxidase (HRP) in 2 h at 37°C in 25 µL 1X Reaction Buffer 2 (50 mM Bis-Tris, 100 mM NaCl, pH 7.0).

Product reconstitution: Dissolve the lyophilized product in 100 µL molecular grade water to make a 1,000 units/mL solution in storage buffer (20 mM Tris-HCl, 200 mM NaCl, pH 7.5). Once reconstituted, store at 4°C for up to 5 days or -20°C for up to 6 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles.

Suggested protocol for protein deglycosylation:

1. Glycoprotein substrate denaturation:
   - 1.1 Mix the following components in a microfuge tube:

Glycoprotein (e.g., RNase B or HRP; user supplied)	50-500 µg
1% SDS (user supplied)	10.0 µL
0.5 M β-Mercaptoethanol or DTT (user supplied)	10.0 µL
10X Reaction Buffer 2 (Cat #BA0601)	10.0 µL
Molecular grade water	to 100 µL final volume

   - 1.2 Heat at 98°C for 10 min. Cool to room temperature.
2. PNGase F-II digestion:
   - 2.1 Mix the following components in a microfuge tube:

Denatured glycoprotein substrate	2-15 µg (in 5 µL or less)
10% Triton X-100 (user supplied)	2.0 µL
10X Reaction Buffer 2 (Cat #BA0601)	2.5 µL
PNGase F-II (Cat #GE0201)	1.0 µL (1 units)
Molecular grade water	to 25 µL final volume

   - 2.2 Incubate at 37°C for 2 h.
   - 2.3 Analyze by SDS-PAGE mobility shift or other method to determine the extent of deglycosylation.

Reference: Sun G, et al. J Biol Chem. 2015 Mar 20;290(12):7452-62. PMID: 25614628

Note: *Reactions may be scaled up to accommodate larger amount and volume of substrate. Titration of enzyme amount and reaction time is recommended for each new substrate. PNGase F-II may remove N-glycans from native glycoproteins at higher enzyme concentration and with longer incubation time. Due to the amount required, PNGase F-II may be visible in gel.*

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