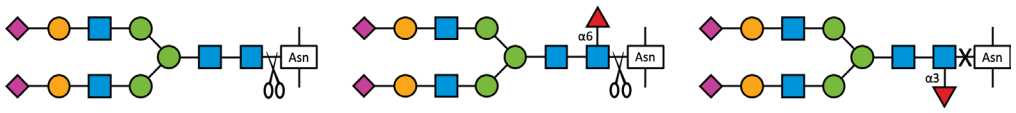
PNGase F contents

Catalog #	Description	Size	M. W.	Purity	pH	Storage
GE0101	PNGase F	4,000 units, lyophilized	37,270	> 95%	7.5-8.5 optimal	-20°C, up to 12 months
BA0501	10X Reaction Buffer 1	1 mL			7.5	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 (Peptide:*N*-Glycosidase F, EC #3.5.1.52, CAS #83534-39-8), cloned from *Elizabethkingia meningosepticum* and expressed in *Escherichia coli* with an *N*-terminal 6xHis tag. It catalyzes the cleavage of asparagine-linked oligosaccharides, except those containing an α1,3-linked core fucose, from glycoproteins and glycopeptides.



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

Unit definition: The amount of PNGase F required to deglycosylate 1 nanomole (15 µg) of denatured RNase B in 1 h at 37°C in 25 µL 1X Reaction Buffer 1 (20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 7.5).

Product reconstitution: Dissolve the lyophilized product in 100 µL molecular grade water to make a 40,000 units/mL solution in 1X Reaction Buffer 1. Once reconstituted, store at 4°C for up to 10 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; 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 1 (Cat #BA0501)	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 digestion:

   2.1 Mix the following components in a microfuge tube:

Denatured glycoprotein substrate	2-15 µg
10% Triton X-100 (user supplied)	2.0 µL
10X Reaction Buffer 1 (Cat #BA0501)	2.5 µL
PNGase F (Cat #GE0101)	1.0 µL (40 units)
Molecular grade water	to 25 µL final volume

   2.2 Incubate at 37°C for 1 h.

   2.3 Analyze by SDS-PAGE mobility shift or other method to determine the extent of deglycosylation.

Reference: Loo T, et al. Protein Expr Purif. 2002 Feb;24(1):90-8. PMID: 11812228.

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

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