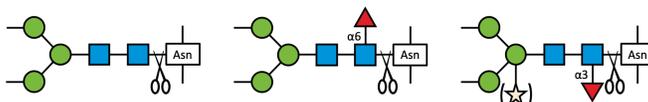


**PNGase F-II contents**

Catalog #	Description	Size	M. W.	Purity	pH	Storage
GE0201-100U	PNGase F-II	100 units, lyophilized	62,300	> 95%	6.5-7.5 optimal	-20°C, up to 12 months
BA0601	10X Reaction Buffer	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*. 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  $\alpha$ 1,3-linked core fucose, PNGase F-II can cleave N-glycans with  $\alpha$ 1,6- or  $\alpha$ 1,3-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  $\mu$ g) of denatured RNase B or 0.1 nanomole (4.5  $\mu$ g) of horse radish peroxidase (HRP) in 2 h at 37°C in 25  $\mu$ L 1X Reaction Buffer (50 mM Bis-Tris, 100 mM NaCl, pH 7.0).

**Product reconstitution:** Dissolve the lyophilized product in 100  $\mu$ L 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 $\mu$ g
1% SDS (user supplied)	10.0 $\mu$ L
0.5 M $\beta$ -Mercaptoethanol or DTT (user supplied)	10.0 $\mu$ L
10X Reaction Buffer (Cat #BA0601)	10.0 $\mu$ L
Molecular grade water	to 100 $\mu$ L 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 $\mu$ g (in 5 $\mu$ L or less)
10% Triton X-100 (user supplied)	2.0 $\mu$ L
10X Reaction Buffer (Cat #BA0601)	2.5 $\mu$ L
PNGase F-II (Cat #GE0201-100U)	1.0 $\mu$ L (1 units)
Molecular grade water	to 25 $\mu$ L 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.

**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.