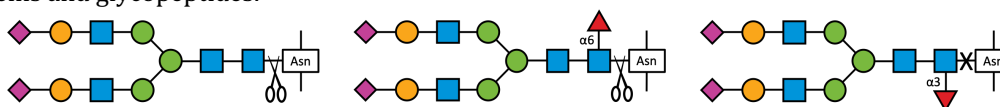


**PNGase F contents**

Catalog #	Description	Size	M. W.	Purity	pH	Storage
GE0101-4KU	PNGase F	4,000 units, lyophilized	37,270	> 95%	7.5-8.5 optimal	-20°C, up to 12 months
GE0101-20KU	PNGase F	20,000 units, lyophilized	37,270	> 95%	7.5-8.5 optimal	-20°C, up to 12 months
BA0501	10X Reaction Buffer	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*. It catalyzes the cleavage of asparagine-linked oligosaccharides, except those containing an  $\alpha$ 1,3-linked core fucose, from glycoproteins and glycopeptides.



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 required to deglycosylate 1 nanomole (15  $\mu$ g) of denatured RNase B in 1 h at 37°C in 25  $\mu$ L 1X Reaction Buffer (20 mM Tris, 50 mM NaCl, 1 mM EDTA, pH 7.5).

**Product reconstitution:** Dissolve the lyophilized product in 100  $\mu$ L molecular grade water to make a 40,000 units/mL (Cat #GE0101-4KU) or a 200,000 units/mL (Cat #GE0101-20KU) solution in 1X Reaction Buffer. 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 $\mu$ 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 #BA0501)	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 digestion:

## 2.1 Mix the following components in a microfuge tube:

Denatured glycoprotein substrate	2-15 $\mu$ g
10% Triton X-100 (user supplied)	2.0 $\mu$ L
10X Reaction Buffer (Cat #BA0501)	2.5 $\mu$ L
PNGase F (Cat #GE0101-4KU or GE0101-20KU)	1.0 $\mu$ L (40 or 200 units)
Molecular grade water	to 25 $\mu$ L 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.

**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.