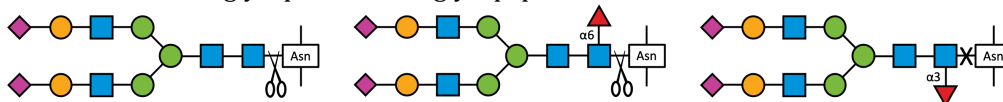


PNGase F contents

| 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 | 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 α 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 μ g) of denatured RNase B in 1 h at 37°C in 25 μ L 1X Reaction Buffer 1 (20 mM Tris, 50 mM NaCl, 1 mM EDTA, pH 7.5).

Product reconstitution: Dissolve the lyophilized product in 100 μ 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 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 μ g |
| 1% SDS (user supplied) | 10.0 μ L |
| 0.5 M β -Mercaptoethanol or DTT (user supplied) | 10.0 μ L |
| 10X Reaction Buffer 1 (Cat #BA0501) | 10.0 μ L |
| Molecular grade water | to 100 μ 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 μ g |
| 10% Triton X-100 (user supplied) | 2.0 μ L |
| 10X Reaction Buffer 1 (Cat #BA0501) | 2.5 μ L |
| PNGase F (Cat #GE0101-4KU or GE0101-20KU) | 1.0 μ L (40 or 200 units) |
| Molecular grade water | to 25 μ 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.

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