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Product Information

PNGase F
from *Elizabethkingia miricola*

Product Number **G5166**
Storage Temperature 2–8 °C

EC 3.5.1.52
CAS RN 83534-39-8
Synonyms: Glycopeptidase F, N-Glycosidase F, Peptide-N^4-(acetyl-β-glucosaminyl)-asparagine amidase, Peptide N-Glycosidase F
Elizabethkingia miricola was formerly known as *Elizabethkingia, Chryseobacterium,* or *Flavobacterium meningosepticum*.

Product Description
PNGase F (Peptide N-Glycosidase F) cleaves asparagine-linked high mannose as well as hybrid and complex oligosaccharides from glycoproteins. It deaminates the asparagine to aspartic acid, but leaves the oligosaccharide intact (see Figure 1). PNGase F will not remove oligosaccharides containing α(1→3)-linked core fucose, commonly found on plant glycoproteins. A tripeptide with the oligosaccharide-linked asparagine as the central residue is the minimal substrate for PNGase F.

Detergent and heat denaturation increases the rate of cleavage up to 100 times. Most native proteins can still be completely N-deglycosylated, but incubation time must be increased. PNGase F will remain active under incubation conditions for at least 72 hours.

PNGase F is isolated from *Elizabethkingia miricola*. It is supplied as a solution in 20 mM Tris HCl, pH 7.5, containing 1 mM EDTA and 50 mM NaCl.

Molecular mass: 36 kDa

pH profile: active in the pH range of 6–10 with an optimal pH of 8.6.

Activity: ≥20,000 units/mg protein and ≥5,000 units/ml

Unit Definition: One unit will catalyze the release of N-linked oligosaccharides from one nanomole of denatured Ribonuclease B in 1 minute at pH 7.5 at 37 °C. One Sigma unit of PNGase F activity is equal to 1 IUB milliunit.

Each lot of PNGase F is tested for contaminating enzyme activity. No protease, exoglycosidase, nor Endoglycosidase F_2 or F_3 activity is detected. Less than 0.01% of Endoglycosidase F_1 can be detected.

Deglycosylation with PNGase F has been used for:

- Amino acid sequence determination
- X-ray crystallography
- Removing heterogeneity due to carbohydrates
- Studying carbohydrate ligand binding
- Removing carbohydrate epitopes from antigens
- Studying the role of glycosylation in protein folding and activity.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
It is recommended to store the product at 2–8 °C. The product remains active for at least 12 months when stored properly. Exposure for several days to ambient temperatures will not reduce activity. **Do Not Freeze.**

Procedure
Procedure for protein deglycosylation

1. Add up to 200 µg of glycoprotein to a microcentrifuge tube. Adjust the final volume to 35 µl with water.

2. Add 10 µl of 250 mM phosphate buffer, pH 7.5, and 2.5 µl of 2% SDS with 1 M 2-mercaptoethanol. Heat at 100 °C for 5 minutes. If SDS or heat denaturation is omitted, increase incubation time to at least 24 hours.

3. Cool, add 2.5 µl of 15% (v/v) TRITON® X-100, and mix. Failure to add TRITON X-100 will result in a 3-fold reduction of PNGase F activity.

4. Add 2.0 µl of the PNGase F solution to the reaction. Incubate for 3 hours at 37 °C. Monitor cleavage by SDS-PAGE.

References

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4. Tarentino, A.L., and Plummer, T.H., Methods in Enzymology, **230**, 44-57 (1994).
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Figure 1.
Specificity of PNGase F