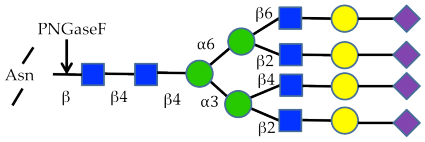
Product Specification Sheet

Product Name: PNGase F (EC 3.5.1.52)
Catalog Number: FSB0002
Lot Number: 031113

Description

PNGase F, peptide N-glycosidase F, is a recombinantly expressed endoglycosidase derived from *Flavobacterium meningosepticum* that cleaves the β-aspartylglucosamine bond between the N-Acetylglucosamine (GlcNAc) and asparagine linkage of N-linked oligosaccharides in glycoproteins (1).



Product Information

Quantity:	10 ug
Specific Activity:	>100 U/mg
Purity:	>95% by SDS-PAGE
Molecular Weight:	34.8 kDa
Ext. coefficient:	2.01 (1 mg/mL at 280 nm in H_2O)
Theoretical PI:	8.39
Storage:	**Buffer:** (20 mM Tris – pH 7.5, 50 mM NaCl, 0.5 mM EDTA) **Temperature:** 2-8°C. Avoid multiple freeze/thaw cycles. **Stability:** 1 year
Unit Definition:	One unit is defined as the amount of enzyme required to catalyze the release of >95% N-linked oligosaccharides from 60 µmoles of denatured ribonuclease B in 1 hour at 37 °C, pH 7.5. One micromolar unit of PNGase F activity is equal to 1,000 nanomolar units (IUB milliunits).

For research use only. Not for use in humans.

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Activity Assay

1. Added 1 μL of 10X Denaturing Solution (5% SDS, 0.4 mM DTT) to 9 μL of substrate solution (containing 1-20 μg of glycoprotein of interest)

2. Incubate substrate solution from step 1 for 5 minutes at 99°C then put back on ice.

3. Added 2 μL of 15% NP-40 solution, 2 μL of 10X Deglycosylation Reaction Buffer (0.5 M sodium phosphate – pH 7.5), 4 μL H_2O, and 2 μL PNGase F to denatured substrate. Include a control reaction by replacing the PNGase F with H_2O.

4. Incubate reaction for 60 minutes at 37°C.

5. Analyze reaction by running treated and untreated reactions in separate lanes on SDS-PAGE gel. Proteins that have been deglycosylated will have increased mobility due to reduction in molecular weight.

References

1. Maley, F. *et al.* (1989) Anal. Biochem. **180**:195.

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