PNGase F (with His-tag, Glycerol Free)

REF: EG23304-S/M

Storage Condition

4℃

Components

Component	EG23304S	EG23304M
PNGase F (Glycerol Free) (500 U/μl)	30 μl	150 μl
10× Denaturing Buffer	150 μl	750 μl
10× PNGase F Buffer	200 μl	1 ml
10% NP-40	200 μl	1 ml

Description

PNGase F (Peptide N-Glycosidase F) is a high effective amidase derived from *Elizabethkingia miricola*. It cleaves asparagine-linked high mannose as well as hybrid and complex oligosaccharides from glycoproteins. The cleavage site of PNGase F is the amide bond between the N-acetylglucosamine (GlcNAc) inside the protein and the asparagine residue, while converting the asparagine residue in the digested protein to aspartic acid.

This product is expressed through recombinant expression in *E.coli*, and it contains a His tag. It does not contain glycerol and is commonly used for deglycosylation of antibodies and related proteins. Avoid repeated freeze-thaw cycles during use.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 μg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 μl.

Heat Inactivation

Incubation at 75℃ for 10 minutes.

Quality Control Assays

Protein Purity

The protein is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Glycosidase and Protease Activity

No detectable activity of endoglycosidases F1, F2, or F3; no detectable protease activity.

Protocol

1. Deglycosylation under denaturing conditions

① Mix 1~20 μg of glycoprotein, 1 μl of 10× Denaturing Buffer, and ddH_2O (if necessary) to a total volume of 10 μl.

Note: The 10× Denaturing Buffer may have white precipitates when stored at low temperatures. Prior to use, dissolve the precipitate by warming it at 37℃ .

② Heat the reaction mixture at 100℃ for 10 minutes to denature the glycoprotein, then cool on ice and centrifuge for 10 seconds.

③ Add 2 μl of 10× PNGase F Buffer, 2 μl of 10% NP-40, and add ddH_2O to make a total reaction volume of 20 μl.

④ Add 1~2 μl of PNGase F, mix gently, and incubate at 37℃ for 1~3 hours.

2. Deglycosylation under non-denaturing conditions

① Mix 1~20 μg of glycoprotein, 2 μl of 10× PNGase F Buffer, and ddH_2O (if necessary) to a total volume of 20 μl.

② Add 2~5 μl of PNGase F, mix gently.

③ Incubate at 37℃ for 4~24 hours.

Removal of PNGase F

This product carries a 6× His-tag. After the deglycosylation reaction, PNGase F can be removed by affinity chromatography.

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