PNGase F, His-tag

Cat #: C-BSM3303

Size: 15000 U / 75000 U

Storage: -20°C

Components

Components	Size 1 (15000 U)	Size 2 (75000 U)
PNGase F (500 U/μl)	30 μl	150 μ
10× Denaturing Buffer	150 μl	750 μl
10× PNGase F Buffer	200 μl	1 ml
10% NP-40	200 μl	1 ml

Product Introduction

PNGase F (Peptide N-glycosidase F) is derived from Elizabethkingia miricola and is an efficient amidase that can cleave high-mannose, hybrid, and complex oligosaccharide glycoproteins linked by asparagine. The cleavage site of PNGase F is the amide bond between the N-acetylglucosamine (GlcNAc) and the asparagine residue on the inside of the glycoprotein, while converting the asparagine residue to aspartic acid. This product is recombinantly expressed in Escherichia coli with a His tag and contains 50% glycerol. It is commonly used for deglycosylation of antibodies and related proteins.

Enzyme Activity Definition

One unit (U) of enzyme activity refers to the amount of enzyme required to remove more than 95% of carbohydrates from 10 μg denatured RNase B in a 10 μl reaction system at 37°C for 1 hour.

Inactivation conditions:

Incubation at 75°C for 10 minutes.

Quality Control

Protein Purity Detection:

The product has been tested by SDS-PAGE, and the purity is ≥95%.

Glycosidase and protease activity detection:

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

Instructions for use:

1. **Deglycosylation under denaturing conditions:**

① Mix 1-20 μg glycoprotein, 1 μl 10× Denaturing Buffer, and ddH2O (if necessary) to a total volume of 10 μl.

Note: The 10× Denaturing Buffer may have white precipitate when stored at low temperature. It can be dissolved by incubating at 37°C before use.

② Denature the glycoprotein by incubating at 100°C for 10 minutes, then cool on ice and centrifuge for 10 seconds.

③ Add 2 μl 10× PNGase F Buffer, 2 μl 10% NP-40, and supplement with ddH2O to a total reaction volume of 20 μl.

④ Add 1-2 μl of PNGase F, mix gently, and incubate at 37°C for 1-3 hours.

2. **Deglycosylation under non-denaturing conditions:**

① Mix 1-20 μg glycoprotein, 2 μl 10× PNGase F Buffer, and ddH2O (if necessary) to a total volume of 20 μl.

② Add 2-5 μl of PNGase F, mix gently.

③ Incubate at 37°C for 4-24 hours.

Removal of PNGase F:

This product contains a His tag, and after the reaction, PNGase F can be removed by affinity chromatography using a different tag from the target glycoprotein.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.