PNGase F, Recombinant (Expressed in *E.coli*)

Cat. No. : RK20565

Product components

Components	Component number	Concentration	Size-1 20,000 U	Size-2 100,000 U
PNGase F, Recombinant (Expressed in *E.coli*)	RM02961	500,000 U/mL	40 μL	200 μL
10X Denaturing Buffer	RM02958	10X	1 mL	1 mL
10X Sodium Phosphate	RM02959	10X	1 mL	1 mL
10% NP-40	RM02960	10%	1 mL	1 mL

Product Description

Peptide N-glycosidase F (PNGase F) is a glycosylpeptidase with a theoretical molecular weight of 35.9 kD that can cleave asparagine-linked high mannose, hybrid, and complex oligosaccharides from glycoproteins. PNGase F cleaves the amide bond between innermost N-acetylglucosamine (GlcNAc) and asparagine residues of the glycoprotein, converting asparagine to aspartic acid in the process. This product is commonly used for the deglycosylation of antibodies and glycoproteins.

Product Source

Cloned from *Elizabeth Mirikola* (*Chryseobacterium miricola*) and expressed in *E. coli,* this protein includes a polyrecombinant amino acid tag.

Storage

Store at -20℃

Unit Definition

One unit is defined as the amount of enzyme required to remove 95% of carbohydrates from 10 μg of denatured RNase B at 37℃ for 1 hr in a total reaction volume of 10 μL.

Reaction Conditions

50 mM Sodium phosphate pH 7.5 @ 25℃, incubate at 37℃.

10X Denatured buffer

5% SDS, 400 mM DTT

10X Sodium phosphate

0.5M Sodium phosphate pH 7.5 @ 25℃

Storage Conditions

20 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, 50% Glycerol, pH 7.5 @ 25℃

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Precautions

1. This product is for scientific research purposes only.
2. Under denaturing conditions, it is more conducive to removing N-glycan chains. Under non-denaturing conditions, it is recommended to increase the amount of enzyme and extend the cleavage time to achieve the best cleavage effect.
3. The concentration of proenzyme is high, so it is recommended to dilute it before use.
4. Avoid repeated freeze-thaw cycles.
5. The experimental system can be scaled up proportionally as needed.
6. PNGase F can be removed using Ni column chromatography,or cation exchange.

Operation Description

1. Digest glycoprotein with PNGase F (denaturing conditions)

Components	10 μL Reaction
glycoprotein	0-10 μg
10X Denaturing Buffer	1 μL
ddH_2O	To 7 μL
Denaturation at 100°C for 10 min, cooling on ice, centrifugation for 10 s	
10% NP-40	1 μL
10X Sodium Phosphate	1 μL
PNGase F, Recombinant (Expressed in *E.coli*) (Dilute to 1-5 U/μL)*	1 μL
37°C for 1 hr	

2. Digest glycoprotein with PNGase F (non-denaturing conditions)

Components	10 μL Reaction
glycoprotein	0-10 μg
10X Sodium Phosphate	1 μL
ddH_2O	To 9 μL
PNGase F, Recombinant (Expressed in *E.coli*) (Dilute to 1-10 U/μL)*	1 μL
37°C for 4-24 hr	

*Note1: When the system volume is small, PNGase F can be appropriately diluted with buffer (50 mM PB pH 7.5@25°C).

Note2: Due to the difference in protein structure and glycosylation modification, the removal efficiency of N-glycoside may vary. Adjust the amount of PNGase F and reaction time as needed to achieve the best experimental results.

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