MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Recombinant PNGase F

Product Number: PSE109

Shipping and Storage

1. Storage temperature: -20±5℃ for storage
2. Validity period: 2 years.

Component

Component	PSE109
PNGase F	30μL
10×Buffer 1	1mL
10×Buffer 2	1mL
NP-40	1mL

Description

N-glycosidase F (PNGase F) is an effective amidase produced by recombinant Escherichia coli with cloned genes encoding the enzyme. It can cleave the N-sugar chain on glycoproteins, helping to generate carbohydrate free peptides and oligosaccharides with di-N-acetyl chitosan units. N-glycosidase F is the most effective method for removing almost all N-sugar chains from glycoproteins. The product can remove all complex, heterozygous, and high mannose sugar chains from antibodies and their fusion proteins, but if there are alpha 1,3-linked fucose residues in the core structure (often expressed in plant and insect cells as immunoglobulins), they cannot be cleaved. Obtaining accurate N-sugar chain distribution in the shortest possible time is crucial for effective process control. N-glycosidase F is an optimized reagent that can rapidly deglycosylate antibodies and fusion proteins within a few minutes. All N-sugar chains can be released quickly and without preference, and can be directly subjected to downstream chromatography or mass spectrometry analysis to quickly determine the glycosylation of antibodies.

Active definition

Unit enzyme activity is defined as the amount of enzyme required to remove over 95% of sugar chains from 10μg denatured RNase B within 1 hour in a 10μL reaction system at 37℃.

Protocol

1. Reaction conditions under denaturing conditions:

For some glycosylated antibodies, it is necessary to denature the sample before complete deglycosylation. The steps are as follows:

1.1. Dissolve 1-20μg of antibodies or glycoproteins in deionized water, dilute to 9μL with deionized water, and then add 1μL of 10 × Buffer 2, resulting in a total volume of 10μL.

1.2. Incubate at 100℃ for 10 minutes, then cool on ice and centrifuge for 10 seconds.

1.3. Add 2μL of 10×Buffer 1, 2μL of 10% NP-40, and 6μL of deionized water, resulting in a final volume of 20μL.

1.4. Add 1μL of PNGase F, gently blow and mix well.

1.5. Incubate at 37℃ for 1 hour.

1.6. The simplest method to evaluate the degree of deglycosylation is to observe the migration changes of the target protein through SDS-PAGE gel electrophoresis.

2. Reaction conditions under non denaturing conditions:

2.1. Dissolve 1-20μg of antibodies or glycoproteins in deionized water, add 2μL of 10×Buffer 1, and the final volume is 20μL.

2.2. Add 2-5μL of PNGase F, gently blow and mix well;

2.3. Incubate at 37℃ for 4-24 hours.

Note: Under denaturing conditions, most substrates can undergo better deglycosylation. Under non denaturing conditions, it may be necessary to increase the amount of PNGase F and prolong the incubation time.

2.4. The simplest method to estimate the degree of glycosylation is to observe the migration changes of the target protein through SDS-PAGE gel electrophoresis.