PNGase F-Fast (Glycerol Free)

Cat.No.: HYI153

Product Description

PNGase F is the most effective enzymatic method for removing almost all N-linked oligosaccharides from glycoproteins. PNGase F is an amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins. It is very important for effective process control to obtain the accurate distribution of N-glycans in a short time. Generally, it takes several hours to hydrolyze and release the N-glycans of the antibody with PNGase F, and then carry out glycan derivatization and HPLC or mass spectrometry analysis. Especially, incomplete deglycosylation can lead to biased results, which will not represent the correct composition of the therapeutic antibody. PNGase F-Fast is an improved reagent, which can release the N-glycans of therapeutic antibody in a few minutes. All N-glycans can be released quickly without bias, and are ready for downstream chromatography or mass spectrometry analysis, so as to determine the glycoprofile of antibodies quickly.

Components

Components	Size
PNGase F-Fast (Glycerol Free)	50 µl
5 × PNGase F-Fast buffer	1000 µl

Specification

Appearance	Colorless clear liquid
Exoglycosidase	No activity could be detected
Endoglycosidase F1	No activity could be detected
Endoglycosidase F2	No activity could be detected
Endoglycosidase F3	No activity could be detected
Endoglycosidase H	No activity could be detected
Protease	No activity could be detected

Properties

EC number	3.5.1.52 (Recombinant from microorganism)

Molecular weight	35 kDa (SDS-PAGE)
Isoelectric point	8.14
Optimum pH	7.0-8.0
Optimum temperature	65 °C
Substrate specificity	Cleaving glycosidic bonds between GlcNAc and asparagine residues
Cleavage sits of IgG	Deglycosylation and leaving the oligosaccharide intact
Activators	DTT
Inhibitor	SDS
Storage temperature	-25～-15 ℃
Heat Inactivation	A 20 µL reaction mixture containing 1 µL of PNGase F is inactivated by incubation at 75 °C for 10 minutes.

Applications

The enzyme is used for rapid deglycosylation of glycosylated antibody, and the purified sugar chain can be used for subsequent mass spectrometry to identify the glycotype of antibody.

Storage

Transport Condition: -25~-15℃

Storage condition: -25~-15℃； the freezing and thawing times of the product are less than 5 times. It is recommended to store the product in small doses after thawing for the first time. After thawing for short term use, it can be stored at 2~8℃, and the recommended time is not more than 30 days.

Reaction conditions

One-step Protocol

1. Dissolve 100 µg of antibody with deionized water to make a volume of 16 µl.
2. Add 4 µl 5×PNGase F-Fast buffer and mix.
3. Add 1 µl PNGase F-Fast and mix.
4. Incubate reaction at 50 °C for 10 min.
5. Prepare N-glycans for derivatization for downstream analysis.

Two-step Protocol

Some antibodies require a preheating step for efficient deglycosylation.

1. Dissolve 100 µg of antibody with deionized water to make a volume of 16 µl.
2. Add 4 µl 5×PNGase F-Fast buffer and mix.
3. Incubate at 80 °C for 2 min and cool on ice.
4. Add 1 µl PNGase F-Fast and mix.
5. Incubate reaction at 50 °C for 10 min.
6. Prepare N-glycans for derivatization for downstream analysis.