SmartEnzymes™

GENOVIS

PNGase F

Immobilized

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

PNGase F Immobilized Microspin 5×0.2mg (G1-PF6-010)
Process 5×0.2 mg glycoprotein

PNGase F Immobilized Microspin 10×0.2mg (G1-PF6-020)
Process 10×0.2 mg glycoprotein

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-G1-PF6

Immobilized Enzyme for Hydrolysis of N-glycans in Spin Columns

PNGase F (Peptide N-glycosidase F) is a glycoamidase hydrolyzing the amide bond between the polypeptide asparagine and the innermost GlcNAc of all mammalian asparagine-linked complex, hybrid, or high-mannose oligosaccharides.

The PNGase F Immobilized spin columns contain the PNGase F enzyme covalently coupled to agarose beads, for hydrolysis of N-glycans with reduced enzyme interference in the final sample. The activity of PNGase F on some glycoproteins can be slow or inhibited due to steric hindrance – longer incubation times or denaturation of the glycoprotein may in these cases be required.

PNGase F is derived from *Elizabethkingia meningoseptica* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 36 kDa.

CONTENT AND STORAGE

The PNGase F Immobilized columns contain sufficient material to hydrolyze Fc N-glycans on 0.2 mg glycoprotein per column. The resin is supplied in 20% ethanol with no preservatives added.

PNGase F Immobilized is shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the product!**

PNGase F Immobilized is for R&D use only.

QUALITY CONTROL

PNGase F Immobilized is tested to meet the specifications and lot-to-lot consistency.

PNGase F Immobilized is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

YOU MIGHT ALSO BE INTERESTED IN

GlycINATOR™
Hydrolysis of all types of Fc N-glycans

OglyZOR™
Hydrolysis of core 1 O-glycans

GalactEXO®
Hydrolysis of β1-3,4 galactose

GalNAcEXO®
Hydrolysis of α-linked GalNAcs

OmniGLYZOR®
Hydrolysis of N- and mucin-type O-glycans

Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).
- PNGase F Immobilized is intended for use under native conditions.

Additional Materials Required

- Reaction buffer: TBS pH 8.6.[1]
- Microcentrifuge tubes (1.5-2 ml).
- Thermal mixer compatible with microcentrifuge tubes.

1. Optimizations may be required if a reaction buffer other than the recommended is used.

Hydrolysis of N-glycans in Spin Columns

Sample Preparation
Prepare the glycoprotein in 100-200 µl reaction buffer per column. Recommended amount of glycoprotein is 0.2 mg per column.

1. Equilibration

1.1 Break off the bottom cap of the column (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.

1.2 Centrifuge at 200 × g for 1 min to remove the storage solution. Discard the flow-through.

1.3 Equilibrate the column by adding 300 µl reaction buffer and centrifuge at 200 × g for 1 min. Discard the flow-through.

1.4 Perform step 1.3 two additional times.

1.5 Insert the bottom cap.

2. Enzymatic Reaction

2.1 Add the glycoprotein to the column (0.2 mg in 100-200 µl reaction buffer).

2.2 Seal the column with the lid. Leave the lid slightly open to prevent excess pressure from building up in the column.

2.3 Fully suspend the media. Do not invert the column (to avoid resin sticking to the lid).

2.4 Incubate the column in a thermal mixer at 37°C with sufficient mixing to keep the resin in suspension (e.g. 600-900 rpm depending on the instrument) for 1 h to overnight[2].

3. Collection of Processed Material

3.1 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

3.2 Centrifuge at 1 000 × g for 1 min to collect the processed material.

4. For Maximum Recovery of the Sample

4.1 Insert the bottom cap.

4.2 Add 100 µl reaction buffer.

4.3 Seal the column with the lid and make sure the media is fully resuspended.

4.4 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

4.5 Centrifuge at 1 000 × g for 1 min to collect the processed material.

4.6 Pool the collected fractions, including the sample from step 3.2.

2. Longer incubation times may be required depending on the glycoprotein.

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