



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

PNGaseF (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 PNGaseF Immobilized spin columns contain the PNGaseF enzyme covalently coupled to agarose beads, for hydrolysis of N-glycans with reduced enzyme interference in the final sample. The activity of PNGaseF 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.

PNGaseF 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 PNGaseF 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.

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

PNGaseF Immobilized is for R&D use only.

QUALITY CONTROL

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

PNGaseF 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 β 1-3,4 galactose

GaINAcEXO™

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).
- PNGaseF 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 μ l 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 \times g for 1 min to remove the storage solution. Discard the flow-through.
- 1.3 Equilibrate the column by adding 300 μ l reaction buffer and centrifuge at 200 \times 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 μ l 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 overnight².

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 \times 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 μ l 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 \times 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.

USA & Canada

Genovis Inc.

245 First Street, Suite 1800, Cambridge, MA 02142, USA

Phone: 1-855-782-0084 (toll free)

Fax: 1-858-524-3006

EMEA & Asia

Genovis AB

Box 4, SE-24421 Kävlinge, Sweden

Phone: +46 46 10 12 30

Fax: +46 46 12 80 20

support@genovis.com

www.genovis.com

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