Immobilized

PNGase F

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INSTRUCTIONS FOR PRODUCTS

Immobilized PNGase F Microspin 5 columns
Deglycosylation to 5 × 0.2 mg glycoprotein (G1-PF6-010)

Immobilized PNGase F Microspin 10 columns
Deglycosylation of 10 × 0.2 mg glycoprotein (G1-PF6-020)

Quick Guide

- The Quick Guide (p. 3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 6).
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and slightly open the lid.

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Quick Guide

1 Equilibration

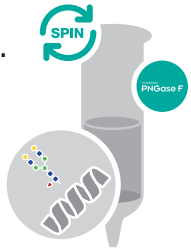
- Equilibrate the column with 3 × 300 µl reaction buffer. Centrifuge at 200 × g for 1 min.

2 Deglycosylation

- *Native reaction conditions:* Add the glycoprotein to the Immobilized PNGase F column and cap the column. Incubate in a thermal mixer at 37°C with 600-900 rpm mixing for 1 h to overnight[1].

3 Collection

- Centrifuge at 1000 × g for 1 min to collect the deglycosylated protein.
- For maximum recovery, add 100 µl reaction buffer, resuspend media and centrifuge at 1000 × g for 1 min.



PRODUCT DESCRIPTION

Immobilized PNGase F is a resin with PNGase F (Peptide N-glycosidase F) covalently coupled to agarose beads for removal of N-glycans on antibodies, fusion proteins and other N-glycosylated proteins. The enzyme is expressed in *E. coli* from a recombinant gene derived from *Elizabethkingia meningoseptica*.

PNGase 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. During the reaction, the asparagine residue from which the glycan is removed is deamidated to aspartic acid. The released oligosaccharide is left intact and can be used for further analysis. Removal of N-glycans is widely used for sample preparation for MS analysis – to reduce the protein heterogeneity and enable released glycan analysis – and to study the functional role of the N-glycan.

The glycoprotein sample is incubated with the Immobilized PNGase F resin in a spin column for 1 h to overnight using native reaction conditions.

The deglycosylated glycoprotein is then easily collected by a centrifugation step. The activity of PNGase F on some glycoproteins can be slow or inhibited due to steric hindrance. Longer incubation times may in these cases be required. Some glycoproteins cannot be fully deglycosylated with PNGase F under native conditions.

Content and Storage

Immobilized PNGase F Microspin columns each contain sufficient material to remove N-glycans from 0.2 mg glycoprotein. The resin is supplied in 20% EtOH with no preservatives added.

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

Immobilized PNGase F Microspin is for R&D use only.

DETAILED PROTOCOL

Equipment Required

- Centrifuge for microcentrifuge tubes
- Thermal mixer compatible with microcentrifuge tubes

Additional Materials Required

- Reaction buffer: TBS pH 8.6[2]
- Collection tubes: Microcentrifuge tubes (1.5-2 ml)

Deglycosylation using Immobilized PNGase F is performed under native conditions.

Deglycosylation using Native Reaction Conditions

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

- Break off the bottom cap of the column (save the cap) and place the column in a collection tube. Loosen the lid.
- Centrifuge at 200 × g for 1 min to remove the storage solution.
- Equilibrate the column by adding 300 µl reaction buffer and centrifuge at 200 × g for 1 min.
- Repeat the equilibration step an additional two times.
- Seal the spin column with the bottom cap.

2 Deglycosylation

- Add the glycoprotein to the column (0.2 mg in 100-200 µl reaction buffer).
- Seal the column with the top lid. Leave the lid slightly loose to avoid pressure build-up in the column during incubation at increased temperature.
- Fully suspend the media. Do not invert the column to prevent resin getting stuck in the lid.
- 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[1].

3 Collection of Deglycosylated Protein

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1 000 × g for 1 min to recover the deglycosylated protein.
- For Maximum Recovery of the Sample:
  - Seal the spin column with the bottom cap.
  - Add 100 µl reaction buffer.
  - Seal the column and make sure the media is fully resuspended.
  - Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
  - Centrifuge the column at 1 000 × g for 1 min to collect the material.
  - Pool the collected fractions.

Notes

1. *Longer incubation times may be required depending on the glycoprotein.*
2. *Optimizations may be required if a reaction buffer other than the recommended is used.*

Quality Control

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

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

Related Products

OglyZOR®
Hydrolysis of core 1 O-glycans

GalactEXO™
Hydrolysis of β1-3,4 galactose

GalNAcEXO™
Hydrolysis of α-linked GalNAcs

GlycINATOR®
Deglycosylation of the IgG Fc domain

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