Immobilized

PNGase F

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STORE CONTENT AT DIFFERENT TEMPERATURES
(See page 5)

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

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

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

Immobilized PNGase F Denaturing Microspin 5 columns
Deglycosylation of up to 5 × 0.2 mg glycoprotein under denaturing conditions (G2-PDK-010)

Immobilized PNGase F Denaturing Microspin 10 columns
Deglycosylation of up to 10 × 0.2 mg glycoprotein under denaturing conditions (G2-PDK-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.

Last revised October 2021

Quick Guide

1 Equilibration

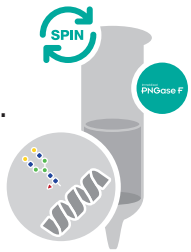
- Equilibrate the column with 3 × 300 µl reaction buffer. Centrifuge at 200 × g for 1 min.

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 650 rpm mixing for 1 h to overnight[1].
- *Denaturing reaction conditions:* Add the denatured glycoprotein to the Immobilized PNGase F column and cap the column. Incubate in a thermal mixer at 50°C with 650 rpm mixing for 15-60 min[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 or

15-60 min using denaturing 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 or denaturation of the glycoprotein may in these cases be required.

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!

*Rapi*Gest™ SF should be stored at room temperature. Once reconstituted in high purity water or a buffer (pH 7-10) the solution is stable for **one week** when stored at +2-8°C.

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

***Rapi*Gest™ SF Surfactant included in Immobilized PNGase F Denaturing**
*Rapi*Gest™ SF Surfactant is a trademark of Waters Corporation.

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)

For preparation of denatured samples for MS analysis:

- DTT
- Formic Acid
- 8 M guanidine hydrochloride pH 8.5 buffered aqueous solution, or equivalent

Depending on the character of the glycoprotein and the following application, the PNGase F deglycosylation can be performed using either native or denaturing reaction conditions. Some glycoproteins require denaturing for full deglycosylation.

Deglycosylation using Native Reaction Conditions

Sample Preparation

- Prepare the glycoprotein in 100-200 µl reaction buffer per column. Max 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 (max 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 650 rpm mixing 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.

Deglycosylation using Denaturing Reaction Conditions

Sample Preparation

- Prepare the glycoprotein in 90-180 µl reaction buffer per column. Max amount of glycoprotein is 0.2 mg per column.
- Reconstitute the content of the *Rapi*Gest SF vial in 20 µl reaction buffer to obtain a 5% solution. Avoid pipetting up-and-down to avoid the formation of foam.
- Add 5% *Rapi*Gest SF solution to the glycoprotein solution to a final *Rapi*Gest SF concentration of 0.5%[3].
- Incubate the glycoprotein solution at 90°C for 5 min to denature the glycoprotein.
- Let the solution cool to room temperature.

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 denatured glycoprotein to the column (max 0.2 mg in 100-200 µl reaction buffer with 0.5% *Rapi*Gest SF).
- 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 50°C with 650 rpm mixing for 15-60 min[1].

❸ 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.

Guidelines for Preparation of Denatured Samples for MS Analysis

To enable MS analysis of denatured samples, *Rapi*Gest SF needs to be removed as follows.

Reduction (Optional)

- If reduction of the deglycosylated protein is required for the following analysis, add DTT to a final concentration of 50 mM. Incubate at 90°C for 5 min.

Acidification – Hydrolysis of *Rapi*Gest SF

- Add formic acid to a final concentration of 1%. Incubate at 37°C for 45 min. Slight cloudiness of the sample may be observed.

Solubilization of the Protein

- Add guanidine hydrochloride solution to a final concentration of 4 M and mix well to solubilize any precipitated protein. Centrifuge the solution at 16 000 × g for 10 min. Transfer supernatant to LC-MS vials for analysis.

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.*
3. *Optimization is needed for deglycosylation at reducing conditions.*

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

Legal and Disclaimers

***Rapi*Gest™ SF Surfactant included in Immobilized PNGase F Denaturing**
*Rapi*Gest™ SF Surfactant is a trademark of Waters Corporation.

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