OmniGLYZOR™

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STORE CONTENT AT DIFFERENT TEMPERATURES
(See page 6)

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

OmniGLYZOR, 5×50-100µg (G3-OM6-005)
Deglycosylation of 5×50-100µg glycoprotein

OmniGLYZOR, 10×50-100µg (G3-OM6-010)
Deglycosylation of 10×50-100µg glycoprotein

Quick Guide

- The Quick Guide (p.3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 8).
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and slightly open the lid.
- If denaturing conditions are needed for PNGase F, an additional step is performed with lyohilized PNGase F in the presence of *Rapi*Gest™ SF Surfactant*. Lyophilized PNGase F and *Rapi*Gest™ SF are included in OmniGLYZOR.

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Quick Guide

1 Equilibration

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

2 Deglycosylation

- Add the glycoprotein to the column and cap the column. Incubate in a thermal mixer at 37°C with 650-850 rpm[1] mixing for 1-4 h[2].

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

OmniGLYZOR contain a resin with a mixture of immobilized enzymes covalently coupled to agarose beads for removal of N- and mucin-type O-glycans on antibodies, fusion proteins and other glycosylated proteins. The enzymes are recombinantly expressed in *E. coli*.

OmniGLYZOR contain enzymes necessary for the removal of N- and simple O-linked glycans (sialyl core 1 and Tn antigen (α-GalNAc)).

The following activities are included:

- PNGase F
- O-Glycosidase
- Sialidase
- α-GalNAcase

Removal of glycans is widely used to reduce heterogeneity to facilitate analysis of glycoproteins by for example mass spectrometry. Deglycosylation

can also be used to study the functional role of the glycans.

The glycoprotein sample is incubated with the OmniGLYZOR Microspin column for 1-4 h under native reaction conditions. The deglycosylated glycoprotein is then easily collected by a centrifugation step.

Certain N-glycosylation sites are poorly or not at all accessible to PNGase F unless the substrate protein is denatured. Should there be any N-glycans left after incubation on the OmniGLYZOR Microspin column, they can be removed by an additional deglycosylation step under denaturing conditions using the lyophilized PNGase F and *Rapi*Gest SF surfactant* included in the kit. The enzymes hydrolyzing the O-glycans do not benefit from denaturation which is why the OmniGLYZOR Microspin columns should only be used under native conditions.

Content and Storage

Reagents included in OmniGLYZOR:

- OmniGLYZOR Microspin columns (5 x 50-100 µg or 10 x 50-100 µg)
- Lyophilized PNGase F (1000 units, 1 vial)
- *Rapi*Gest SF Surfactant (1 mg, 1 or 2 vials)

OmniGLYZOR Microspin columns contain sufficient material to remove N- and O- glycans from 50-100 µg glycoprotein. The resin is supplied in 20% EtOH with no preservatives added. OmniGLYZOR Microspin columns are shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the microspin columns!**

PNGase F is supplied lyophilized in 50 mM HEPES buffer pH 7.5, with no preservatives added. PNGase F is shipped cold and should be stored at -20°C upon arrival. After reconstitution, PNGase F is stable for at least 1 month at +4-8°C.

*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.

OmniGLYZOR is for R&D use only.

** RapiGest™ SF Surfactant is a trademark of Waters Corporation.*

Use lids and bottom caps during the incubation.

Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).

Equipment Required

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

Additional Materials Required

- Reaction buffer: PBS pH 7.4[3]
- 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

Deglycosylation

Sample Preparation

- Prepare the glycoprotein in 50-100 µl reaction buffer per column. Recommended amount of glycoprotein is 50-100 µg 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 (50-100 µg in 50-100 µl reaction buffer).
- Seal the column with the top lid.

- 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 650-850 rpm[1] mixing for 1-4 h[2].

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.

Additional Deglycosylation with PNGase F under Denaturing Reaction Conditions

Sample Preparation

- Use a part or the entire collected fraction from the OmniGLYZOR column for further deglycosylation under denaturing conditions if necessary.
- 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 0.1-0.2 %.
- Optional Reduction: add DTT to a final concentration of up to 50 mM to reduce disulfide bonds.
- Incubate the glycoprotein solution at 90°C for 5 min to denature the glycoprotein.
- Let the solution cool to room temperature.

1 Prepare PNGase F

- Reconstitute PNGase F in 50 µl ddH_2O to a concentration of 20 units/µl.

2 Add PNGase F

- Add 1 unit PNGase F / 1 µg glycoprotein.

3 Deglycosylation

- Incubate for 30 min at 50°C[2].

Guidelines for Preparation of Denatured Samples for MS Analysis

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 16000 × g for 10 min. Transfer the supernatant to LC-MS vials for analysis.

Notes

1. *The optimal speed of mixing depends on the model of thermal mixer that is used and might be higher than the range given here. The resin must stay in suspension during the entire incubation for optimal performance.*
2. *Incubation times depend on the glycoprotein. Some substrates might need longer incubation, especially substrates with poorly accessible N-glycans might need overnight incubation to be removed under native conditions. Alternatively, perform the additional deglycosylation step under denaturing conditions.*
3. *Optimizations may be required if a reaction buffer other than the recommended one is used.*

Quality Control

OmniGLYZOR is tested to meet the specifications and lot-to-lot consistency.

OmniGLYZOR 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®
Hydrolysis of IgG Fc glycans

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