SmartEnzymes™

GENOVIS

OmniGLYZOR®

Kit

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FOR RESEARCH USE ONLY

Instructions for Use

OmniGLYZOR® Kit Microspin 5×50-100µg (G3-OM6-005)
Process 5×50-100µg glycoprotein

OmniGLYZOR® Kit Microspin 10×50-100µg (G3-OM6-010)
Process 10×50-100µg glycoprotein

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-G3-OM6

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A Mix of Immobilized Enzymes in Spin Columns for Hydrolysis of N- and Mucin-type O-glycans

OmniGLYZOR contains a mixture of immobilized enzymes for fast and efficient removal of N- and simple mucin-type O-glycans on antibodies, fusion proteins and other glycosylated proteins. OmniGLYZOR Kit includes spin columns with OmniGLYZOR Immobilized resin featuring the following enzymatic activities: PNGase F, O-glycosidase, sialidase, and α-GalNAcase.

Certain N-glycosylation sites are only partially accessible – or not accessible at all – to PNGase F unless the substrate protein is denatured. To remove residual N-glycans following incubation on the OmniGLYZOR Microspin column, an additional deglycosylation step can be performed under denaturing conditions using the PNGase F Lyophilized enzyme and *Rapi*Gest™ SF surfactant included in the kit. In contrast, the enzymes used for O-glycan removal do not benefit from denaturation. Therefore, OmniGLYZOR columns should be used exclusively under native conditions.

CONTENT AND STORAGE

OmniGLYZOR Kit contains three components. The product box is shipped cold, and the three components should be stored at different temperatures upon arrival.

- **OmniGLYZOR Immobilized Microspin** is supplied in 20% ethanol with no preservatives added. One spin column contains sufficient material to process 50-100 µg glycoprotein. OmniGLYZOR Immobilized should be stored at +4-8°C upon arrival. **Do not freeze the product!**
- **PNGase F Lyophilized** is supplied lyophilized in 50 mM HEPES buffer pH 7.5, with no preservatives added. PNGase F Lyophilized should be stored at -20°C upon arrival. After reconstitution, PNGase F Lyophilized is stable for at least 1 month at +4-8°C.
- ***Rapi*Gest™* SF surfactant** should be stored at room temperature upon arrival. Once reconstituted in high purity water or a buffer (pH 7.0–10.0), the solution is stable for one week when stored at +2–8°C.

OmniGLYZOR Kit is for R&D use only.

QUALITY CONTROL

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

OmniGLYZOR 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

OglyZOR™
Hydrolysis of core 1 O-glycans

GalactEXO®
Hydrolysis of β1-3,4 galactose

GalNAcEXO®
Hydrolysis of α-linked GalNAcs

GlycINATOR™
Hydrolysis of all types of Fc N-glycans

* *Rapi*Gest™ SF Surfactant is a trademark of Waters Corporation.

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).

Additional Materials Required

- Reaction buffer: PBS pH 7.4[1]
- 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

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

Hydrolysis of N- and Mucin-type O-glycans

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

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 (50-100 µg in 50-100 µl reaction buffer).

2.2 Seal the column with the lid.

2.3 Fully suspend the media. Do not invert the column to prevent resin getting stuck in the lid.

2.4 Incubate the column in a thermal mixer at 37°C with 650-850 rpm[2] mixing for 1-4 h[3].

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 deglycosylated protein.

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 material.

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

2. 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.
3. 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.

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 RapiGest 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.

5. Prepare PNGase F

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

6. Add PNGase F

6.1 Add 1 unit PNGase F/1 µg glycoprotein.

7. Enzymatic Reaction

7.1 Incubate for 30 min at 50°C.[3]

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 16 000 × g for 10 min. Transfer the supernatant to LC-MS vials for analysis.

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