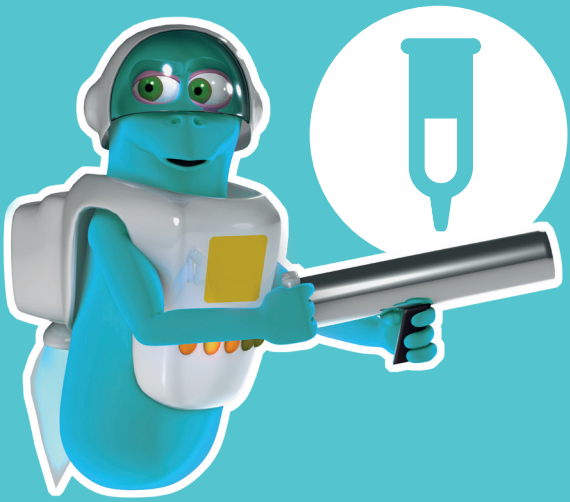
Immobilized

GalNAcEXO™

FOR RESEARCH
USE ONLY

www.genovis.com

STORE AT
+4-8°C



SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

Immobilized GalNAcEXO Microspin 2 × 0.5 mg
Digestion of up to 2 × 0.5 mg glycoprotein (G1-NA6-010)

Immobilized GalNAcEXO Microspin 5 × 0.5 mg
Digestion of up to 5 × 0.5 mg glycoprotein (G1-NA6-025)

Immobilized GalNAcEXO Microspin 10 × 0.5 mg
Digestion of up to 10 × 0.5 mg glycoprotein (G1-NA6-050)

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.

Sample Preparation

- Prepare the glycoprotein in 100-300 µl reaction buffer. Max 0.5 mg glycoprotein per column.

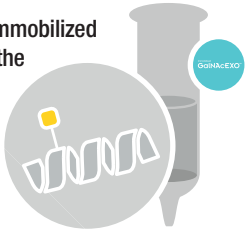
Last revised September 2020

1 Equilibration

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

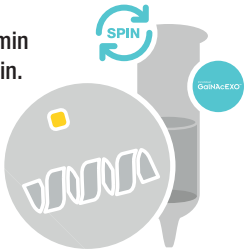
2 Digestion

- Add the glycoprotein to the Immobilized GalNAcEXO column and cap the column. Incubate at room temperature with end-over-end mixing for 2-4 h.



3 Collection

- Centrifuge at 1000 x g for 1 min to collect the digested protein.
- For maximum recovery, add 100 µl reaction buffer, invert and centrifuge at 1000 x g for 1 min.
- Repeat once.



PRODUCT DESCRIPTION

Immobilized GalNAcEXO is a resin with an exo-α-*N*-Acetylgalactosaminidase covalently coupled to agarose beads for efficient hydrolysis of α-*N*-acetylgalactosamine (GalNAc) linked to serine or threonine residues in glycoproteins (Tn antigen). GalNAcEXO has some activity on other terminal α-linked GalNAc. GalNAcEXO hydrolyzes glycoproteins under native conditions and is highly active in a pH range of 6.0 to 7.6. No cofactors or special buffers are required.

Deglycosylated proteins are generated without the enzyme in the final preparation. The glycoprotein sample is incubated with the Immobilized GalNAcEXO resin and the digested glycoproteins are then easily collected by a centrifugation step. The recommended buffer for Immobilized GalNAcEXO is 20 mM Tris pH 6.8[1,2]. The protocol may need optimization regarding buffer compatibility and incubation time for individual glycoproteins.

Content and Storage

Each Immobilized GalNAcEXO Microspin columns contain sufficient material to remove α-linked GalNAc from 0.5 mg glycoprotein. The resin is supplied in 20 % EtOH with no preservatives added.

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

Immobilized GalNAcEXO Microspin is for R&D use only.

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[1,2]: 20 mM Tris pH 6.8
- Collection tubes: Microcentrifuge tubes (1.5-2 ml)

Sample Preparation

- Prepare the glycoprotein in 100-300 µl reaction buffer per column. The maximum amount of glycoprotein is 0.5 mg per column.

Digestion of Glycoprotein on Immobilized GalNAcEXO 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 two times.
- Seal the spin column with the bottom cap.

2 Digestion

- Add the glycoprotein to be digested in a volume of 100-300 µl digestion buffer. Max 0.5 mg glycoprotein per column.
- Seal the column with the top lid.
- Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- Incubate the column with end-over-end mixing at room temperature for 2 to 4 hours.[3]

❸ Collection of Digested Protein

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1000 × g for 1 min to recover the digested glycoproteins.

For Maximum Recovery of the Sample

- Seal the spin column with the bottom cap.
- Add 100 µl reaction buffer[2].
- Seal the column and invert the column a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1000 × g for 1 min to collect the material.
- Repeat once.
- Pool the collected fractions.

Notes

1. Immobilized GalNAcEXO displays high activity in buffers with pH values from 6.0 to 7.6 and over a wide range of ionic strength. Some optimizations might be required if a buffer other than the recommended reaction buffer is used.
2. If the glycoprotein sticks to the resin, a buffer with higher salt concentration can be used in the reaction and/or in the wash steps.
3. Longer incubation times may be required depending on the glycoprotein.

Quality Control

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

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

Related Products

GalactEXO™
For complete removal of β1-3 and β1-4 linked galactoses.

SialEXO®
For complete removal of α2-3, α2-6 and α2-8 linked sialic acids.

OglyZOR®
Specific hydrolysis of core 1 O-glycans disaccharides on native glycoproteins.

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