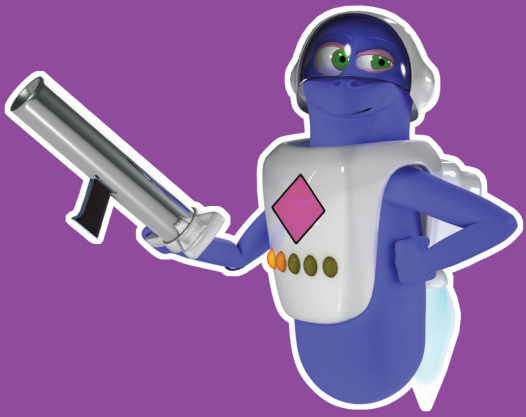
Immobilized

SialEXO®

FOR RESEARCH
USE ONLY
www.genovis.com

STORE AT
+4-8°C



SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

Immobilized SialEXO® Microspin 2 columns (G1-SM6-010)
Desialylation of up to 2 × 0.5 mg glycoprotein

Immobilized SialEXO® Microspin 5 columns (G1-SM6-025)
Desialylation of up to 5 × 0.5 mg glycoprotein

Immobilized SialEXO® Microspin 10 columns (G1-SM6-050)
Desialylation of up to 10 × 0.5 mg glycoprotein

Quick Guide

- The Quick Guide (p. 3) is intended for experienced users. First time users are recommended to follow the detailed protocol (p. 5).
- 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.

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Desialylation – Immobilized SialEXO® Microspin

1 Equilibration

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

2 Digestion

- Add the glycoprotein to the Immobilized SialEXO column and cap the column.
- Incubate at room temperature with end-over-end mixing for 30 min.

3 Collection

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

PRODUCT DESCRIPTION

Immobilized SialEXO is a resin with a mixture of two sialidases covalently coupled to agarose beads for complete removal of sialic acids (α2-3, 2-6 & 2-8, linked) of *O*- and *N*-glycosylated proteins. The enzymes in SialEXO are derived from *Akkermansia muciniphila* and expressed in *E. coli*.

Desialylated proteins are generated without the enzyme in the final preparation. The glycoprotein sample is incubated with the Immobilized SialEXO resin and the desialylated glycoproteins are then easily collected by a centrifugation step. The recommended buffer for Immobilized SialEXO is 20 mM Tris pH 6.8[1,3]. The protocol may need optimization regarding buffer compatibility and incubation time for individual glycoproteins.

Content and Storage

Immobilized SialEXO Microspin columns contain sufficient material each to desialylate 0.5 mg glycoprotein. The resin is supplied in 20% EtOH with no preservatives added.

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

Immobilized SialEXO 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[1,3]: 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. Max amount of glycoprotein is 0.5 mg per column.

Desialylation of Glycoprotein on Immobilized SialEXO® 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 Desialylation

- Add the glycoprotein to be desialylated 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 30 min[2].

3 Collection of Desialylated 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 desialylated glycoproteins.

For Maximum Recovery of the Sample:

- Seal the spin column with the bottom cap.
- Add 100 µl reaction buffer[3].
- 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. *SialEXO displays high activity in buffers at pH 6.5-9.*
2. *Longer incubation times may be required depending on the glycoprotein.*
3. *If the glycoprotein sticks to the resin, a buffer with NaCl can be used in the reaction and/or in the wash steps.*

Quality Control

Immobilized SialEXO is tested to meet specifications and lot-to-lot consistency.

Immobilized SialEXO is tested for absence of microbial contamination with blood agar plates,

Sabouraud dextrose agar plates and fluid thioglycollate medium.

Related Products

SialEXO®
Complete removal of sialic acids from glycoproteins

SialEXO® 23
Removal of 2-3 linked sialic acids from glycoproteins

OpeRATOR®
O-glycan specific endoprotease digesting N-terminally of mucin-type O-glycans

GlycOCATCH®
Enrichment of mucin-type O-glycosylated proteins and peptides

Legal and Disclaimers

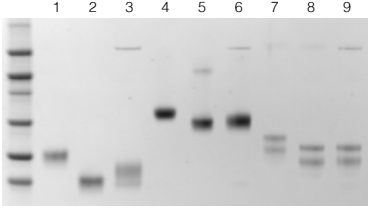
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

OglyZOR®

O-glycosidase Hydrolyzing Core 1 O-glycans

OglyZOR is an O-glycosidase that catalyzes the removal of core 1 and to some extent core 3 type O-linked disaccharides from native glycoproteins.

- Hydrolyzes O-glycans
- Specific for core 1 and to some extent core 3 type O-glycans



Comparison of the enzymatic activities of OglyZOR and SialEXO to commercially available endoglycosidases and sialidases. All incubations (4 h) were performed according to the manufacturers instructions, and the samples were all separated on SDS-PAGE.

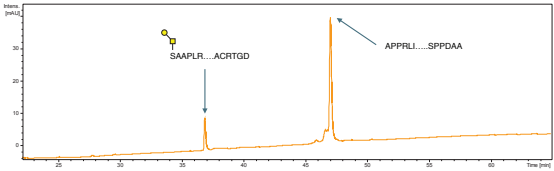
1. TNF receptor
2. + SialEXO and OglyZOR
3. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
4. Etanercept
5. + SialEXO and OglyZOR
6. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
7. Fetuin
8. + SialEXO and OglyZOR
9. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)

OpeRATOR®

O-glycan-specific Endoprotease

OpeRATOR is a novel tool for analysis of mucin-type O-glycans on glycoproteins. The protein binds to O-glycans and digests the amino acid backbone N-terminally of the S/T glycosylation site.

- O-glycan-specific, mucin-type
- Requires O-glycans for activity
- Generates glycopeptides with O-glycans and allows for O-glycan profiling and site-occupancy determination using mass spectrometry.



Erythropoietin (EPO) is a ~30 kDa glycoprotein with one O-glycan site. The protein was used here as a substrate to demonstrate the specific activity of the OpeRATOR protease. OpeRATOR hydrolyzed the protein N- terminally of the serine O-glycan site, and after reduction of disulfide bridges, the resulting two fragments were separated and intact mass was analyzed using a Bruker Impact II ESI QTOF MS.

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