OglyZOR®

FOR RESEARCH
USE ONLY
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STORE AT
-20°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCT

OglyZOR® 2000 units (G2-OG1-020)
Deglycosylation of up to 2 mg O-glycoprotein (core 1)

Last revised Feb 2020

1 Prepare OglyZOR® and SialEXO®

Reconstitute OglyZOR and SialEXO in 50 µl ddH_2O each to a concentration of 40 units/µl.

2 Add SialEXO® followed by OglyZOR®

Add 1 unit SialEXO / 1 µg glycoprotein, followed by 1 unit OglyZOR / 1 µg glycoprotein

3 Deglycosylation

Incubate for 2 -4 h at 37°C.

PRODUCT DESCRIPTION

OglyZOR is an endoglycosidase that catalyzes the removal of core 1 and to a limited extent core 3 O-linked disaccharides from native glycoproteins. OglyZOR is only active on desialylated O-glycans. SialEXO, a mix of two sialidases, for removal of α2-3, α2-6 and α2-8 linked sialic acids, is used together with OglyZOR for efficient removal of the O-linked disaccharides (Gal-GalNAc). SialEXO is included in the box.

OglyZOR enzyme is derived from *Streptococcus oralis* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 227 kDa. SialEXO is derived from *Akkermansia muciniphila* and expressed in *E. coli*. The enzymes in SialEXO contain His-tags and the molecular weights are 43 kDa and 66 kDa, respectively.

Unit Definition

One unit of OglyZOR removes ≥ 90% of O-glycans of 1 µg glycoprotein (TNFR) when incubated together with one unit of SialEXO in 20 mM Tris pH 6.8 at 37 °C for 2 h.

Content and Storage

OglyZOR is supplied lyophilized in TBS pH 7.6.

SialEXO is supplied lyophilized in TBS pH 7.6.

The OglyZOR box is shipped at ambient temperature and the vials should be stored at -20°C upon arrival.

After reconstitution, the enzymes are stable for at least 1 month at +4-8°C.

OglyZOR is for R&D use only.

Additional Materials Required

- Reaction buffer[1]: 20 mM Tris, pH 6.8

Sample Preparation

- Prepare the glycoprotein of interest in reaction buffer in a concentration of 0.1-2 mg/ml.

Deglycosylation

1. **Prepare OglyZOR® and SialEXO®**
   Reconstitute OglyZOR and SialEXO in 50 µl ddH_2O each to 40 units/µl[2].

2. **Add SialEXO®**
   Add 1 unit SialEXO / 1 µg glycoprotein[3].

3. **Add OglyZOR®**
   Add 1 unit OglyZOR / 1 µg glycoprotein[3].

4. **Deglycosylation**
   Incubate for 2-4 h at 37°C.

Notes

1. *The OglyZOR enzyme displays optimal activity in a pH range of 6.5 to 7.5.*
2. *To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).*
3. *A higher enzyme concentration may increase digestion efficiency of individual glycoproteins. This requires optimization.*

Quality Control

OglyZOR and SialEXO are tested to meet the specifications and lot-to-lot consistency.

OglyZOR and SialEXO are tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Related Products

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

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

SialEXO®
Sialidase mix for complete removal of sialic acids

Immobilized SialEXO®
Immobilized SialEXO for complete removal of sialic acids with no enzyme in the final preparation

Legal and Disclaimers

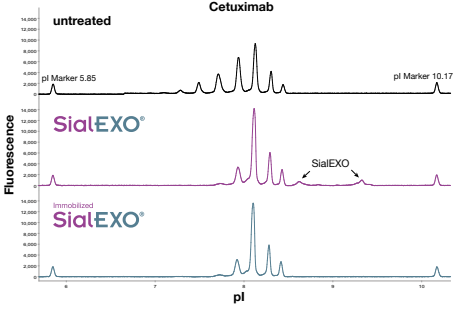
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

SialEXO®

Complete Removal of Sialic Acids

SialEXO is a sialidase mix for complete removal of sialic acids from native glycoproteins.

- Acts on α2-3, α2-6 and α2-8 linkages
- Hydrolyzes sialic acids on both N- and O-linked glycans
- Available in an immobilized format for removal of sialic acids within 30 minutes



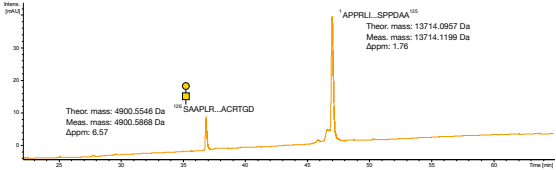
Desialylation of cetuximab using SialEXO and Immobilized SialEXO followed by imaged isoelectric focusing. Data obtained in collaboration with ProteinSimple.

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 peptide backbone N-terminally of the S/T glycosylation sites.

- 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 core 1 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 by Q-TOF MS using ESI.

US & Canada

Genovis Inc.
245 First Street, Suite 1800
Cambridge, MA 02142
USA

Customer service: 617-444-8421
Order phone (toll free): 855-782-0084
Order fax: 858-524-3006
Email: orders.us@genovis.com

EMEA & Asia

Genovis AB
Box 790
SE-220 07 Lund
Sweden

Customer service: +46 46 10 12 30
Order phone: +46 46 10 12 30
Order fax: +46 46 12 80 20
Email: order@genovis.com