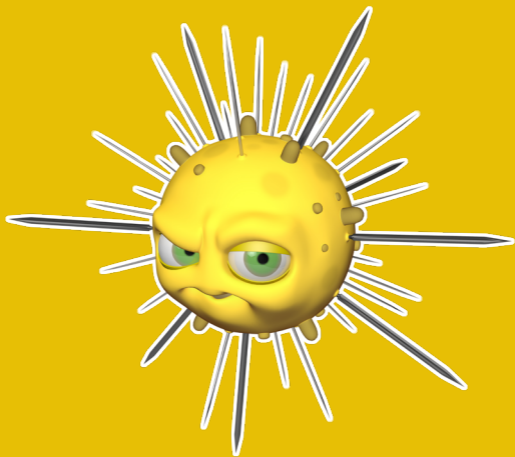


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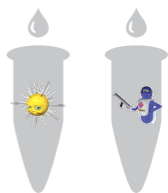
INSTRUCTIONS FOR PRODUCT

(G2-OP1-020)

Digestion of up to 2 mg O-glycoprotein

1 Prepare OpeRATOR[®] and SialEXO[®]

Reconstitute OpeRATOR and SialEXO in 50 μl ddH₂O each to a concentration of 40 units/ μl .



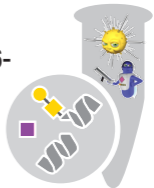
2 Add SialEXO[®] followed by OpeRATOR[®]

Add 1 unit SialEXO followed by 1 unit OpeRATOR / 1 μg glycoprotein.



3 Digestion

Incubate for 2 h to overnight (16-18 h) at 37°C.



PRODUCT DESCRIPTION

OpeRATOR is an endoprotease digesting proteins with mucin-type O-glycosylation. The enzyme binds to O-glycans (core 1) and digests the peptide backbone N-terminally at the site of O-glycosylation (serine and/or threonine). This generates glycopeptides carrying O-glycans at the N-terminal end, and enables O-glycan profiling, O-glycopeptide mapping and site occupancy determination, as well as middle-level approaches using mass spectrometry.

The OpeRATOR enzyme is most active towards sites with asialylated core 1 O-glycans (1). Sialylated sites and sites with core 3 O-glycans are digested with significantly reduced activity¹. SialEXO, a sialidase mix for efficient removal of sialic acids (2-3, 2-6 and 2-8 linked), is included in the OpeRATOR box.

OpeRATOR is derived from *Akkermansia muciniphila* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 42 kDa. Enzymes in SialEXO are also derived from *Akkermansia muciniphila* and expressed in *E. coli*. They contain His-tags and their molecular weights are 43 kDa and 66 kDa, respectively.

Unit Definition

One unit OpeRATOR digests $\geq 90\%$ 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

OpeRATOR is supplied lyophilized in TBS pH 7.6.
SialEXO is supplied lyophilized in TBS pH 7.6.

The OpeRATOR 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.

OpeRATOR is for R&D use only.

DETAILED PROTOCOL

Additional Materials Required

- Digestion buffer²: 20 mM Tris, pH 6.8.

Sample Preparation

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

Digestion of O-glycosylated Proteins with Removal of Sialic Acids

1

Prepare OpeRATOR[®] and SialEXO[®]

Reconstitute OpeRATOR and SialEXO in 50 μ l ddH₂O to 40 units/ μ l³.

2

Add SialEXO[®]

Add 1 unit SialEXO / 1 μ g glycoprotein⁴.

3

Add OpeRATOR[®]

Add 1 unit OpeRATOR / 1 μ g glycoprotein⁴.

4

Digestion

Incubate for 2 h to overnight (16-18 h) at 37°C.

The activity of the OpeRATOR enzyme is significantly reduced if sialic acids are present¹.

Notes

1. Glycoproteins with O-glycosylation core 3 and 2,3 sialyl core 1 are digested poorly. O-glycan sites with Tn-antigen, core 2 and 2,6 sialyl core 1 are not digested at all.
 2. The OpeRATOR enzyme has optimal activity in a pH range of pH 5.5 to 7.5.
 3. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
 4. A higher enzyme concentration may increase digestion efficiency of individual glycoproteins. This requires optimization.
-

Quality Control

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

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

Reference

1. Trastoy, B., Naegeli, A., Anso, I. et al. Structural basis of mammalian mucin processing by the human gut O-glycopeptidase OgpA from *Akkermansia muciniphila*. *Nat Commun* 11, 4844 (2020). <https://doi.org/10.1038/s41467-020-18696-y>

Related Products

GlycOCATCH®

Enrichment of mucin-type O-glycosylated proteins and peptides

OglyZOR®

Hydrolyzes core 1 disaccharide (Gal- β 1-3-GalNAc) from O-glycosylated proteins

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

More Information

Download the OpeRATOR® Application Note

"Determination of O-glycan sites by OpeRATOR® digestion and LC-MS"



<https://www.genovis.com/G2OP1909>

OpeRATOR®

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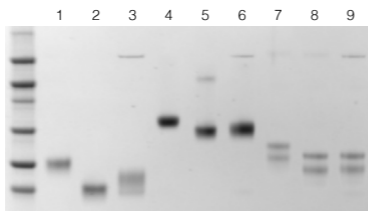
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

OglyZOR®

O-glycosidase Hydrolyzing Core 1 O-glycans

OglyZOR is an O-glycosidase that catalyzes the removal of core 1 O-linked disaccharides from native glycoproteins.

- Efficient removal of the core 1 disaccharide (Gal- β 1-3-GalNAc) from O-glycosylated proteins
- Requires removal of sialic acids using SialEXO (included)



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.

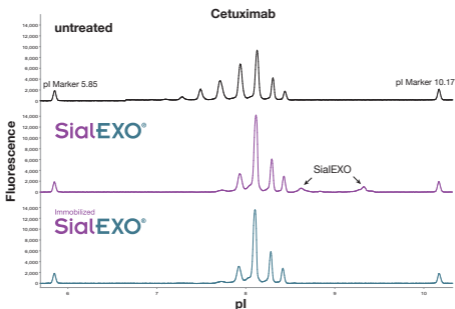
1. TNF receptor
2. + SialEXO and OglyZOR
3. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
4. Etanercept
5. + SialEXO and OglyZOR
6. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
7. Fetuin
8. + SialEXO and OglyZOR
9. + Endoglycosidase (*E. faecalis*) and sialidase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

SialEXO[®]

Complete Removal of Sialic Acids

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

- Acts on 2-3, 2-6 and 2-8 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.



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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
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Customer service: +46 46 10 12 30
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Order fax: +46 46 12 80 20
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