

# OglyZOR<sup>®</sup>

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FOR RESEARCH  
USE ONLY  
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STORE AT  
-20°C



SmartEnzymes<sup>™</sup>

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## INSTRUCTIONS FOR PRODUCT

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**OglyZOR® 2000 units** (G2-OG1-020)

Deglycosylation of up to 2 mg O-glycoprotein (core 1)

## 1 Prepare OglyZOR<sup>®</sup> and SialEXO<sup>®</sup>

Reconstitute OglyZOR and SialEXO in 50  $\mu$ l ddH<sub>2</sub>O each to a concentration of 40 units/ $\mu$ l.



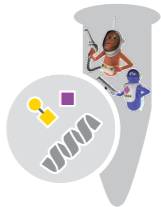
## 2 Add SialEXO<sup>®</sup> followed by OglyZOR<sup>®</sup>

Add 1 unit SialEXO / 1  $\mu$ g glycoprotein, followed by 1 unit OglyZOR / 1  $\mu$ g glycoprotein



## 3 Deglycosylation

Incubate for 2 -4 h at 37°C.



# PRODUCT DESCRIPTION

OglyZOR is an endoglycosidase that catalyzes the removal of core 1 O-linked disaccharides from native glycoproteins. OglyZOR is only active on desialylated O-glycans. SialEXO, a mix of two sialidases, for removal of  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linked sialic acids, is used together with OglyZOR for efficient removal of the O-linked disaccharides (Gal- $\beta$ 1-3-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  $\geq 90\%$  of O-glycans of 1  $\mu$ g 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<sup>1</sup>: 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<sup>®</sup> and SialEXO<sup>®</sup>

Reconstitute OglyZOR and SialEXO in 50  $\mu$ l ddH<sub>2</sub>O each to 40 units/ $\mu$ l<sup>2</sup>.

### 2 Add SialEXO<sup>®</sup>

Add 1 unit SialEXO / 1  $\mu$ g glycoprotein<sup>3</sup>.

### 3 Add OglyZOR<sup>®</sup>

Add 1 unit OglyZOR / 1  $\mu$ g glycoprotein<sup>3</sup>.

### 4 Deglycosylation

Incubate for 2-4 h at 37°C.

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## 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



OglyZOR®

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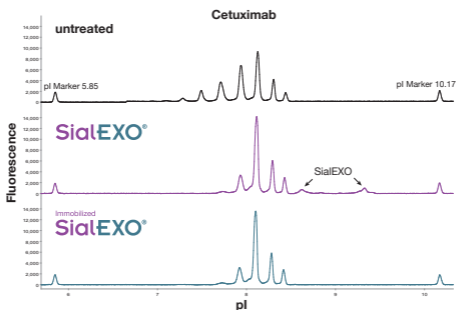
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

## SialEXO®

### Complete Removal of Sialic Acids

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

- Acts on  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 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



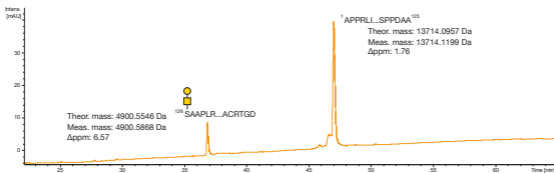
Desialylation of cetuximab using SialEXO and Immobilized SialEXO followed by imaged isoelectric focusing. Data obtained in collaboration with ProteinSimple.

# OpeRATOR<sup>®</sup>

## O-glycan-specific Endoprotease

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

- Specific for mucin-type O-glycans (core 1)
- No activity on unmodified S/T residues
- Generates O-glycopeptides suitable for mapping of glycosylation sites using LC-MS



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 ESI-Q-TOF mass spectrometry.



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