

## INSTRUCTIONS

Version 17.1.2

Instructions for product no:  
G2-OP1-020

2000 units

Digestion of up to 2 mg protein with O-linked glycans

### Content and Storage

The OpeRATOR® box includes:

- 1 vial of OpeRATOR® enzyme (G1-OP1-020) supplied lyophilized in TBS pH 7.6, with no preservatives added.
- 1 vial of SialEXO® (G1-SM1-020) supplied lyophilized in TBS pH 7.6, with no preservatives added.

The vials in the OpeRATOR® box are shipped cold and should be stored at -20 °C upon arrival.  
After reconstitution, the enzymes of the box are stable for 1 month at +4-8 °C.  
OpeRATOR® box is for R&D use only.

### Product Description

OpeRATOR® enzyme is an endoprotease digesting O-glycosylated proteins at the N-terminus of the Ser / Thr glycosylation site. The OpeRATOR® is an O-protease which is active on native glycoproteins. It is highly specific and digests the amino acid backbone only in presence of O-glycans, generating distinct glycopeptides suitable for applications such as O-glycan profiling, O-glycan site determination, O-glycopeptide mapping and middle down approaches. OpeRATOR® is active on O-glycan proteins with sialic acids but the enzymatic activity is higher if the sialic acids are removed. SialEXO® a mix of two sialidases, is for removal of  $\alpha$ 2,3-,  $\alpha$ 2,6- and  $\alpha$ 2,8-linked sialic acids. For convenience, it is included in the box.

OpeRATOR® enzyme is derived from *Akkermansia muciniphila* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 41.8 kDa. Enzymes in SialEXO® is derived from *Akkermansia muciniphila* and expressed in *E. coli*. The enzymes in the SialEXO® contain His-tags and the molecular weights are 42.8 kDa and 65.7 kDa, respectively.

### Unit Definition

One unit OpeRATOR® digests  $\geq$  90% of 1  $\mu$ g glycoprotein (TNF $\alpha$ R) when incubated together with one unit of SialEXO® in 20 mM Tris pH 6.8 at 37 °C for 2 h.

### Quality Control

OpeRATOR® and SialEXO® in the box are tested to meet specifications.

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

### Protocol

#### Additional Materials Required

Digestion buffer<sup>1</sup>: 20 mM Tris pH 6.8

#### Preparation of glycoprotein

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

#### Option 1

##### Digestion of O-glycosylated protein with removal of sialic acids

- Reconstitute SialEXO® in 50  $\mu$ l ddH<sub>2</sub>O<sup>2</sup> to a concentration of 40 units /  $\mu$ l.
- Reconstitute OpeRATOR® in 50  $\mu$ l ddH<sub>2</sub>O<sup>2</sup> to a concentration of 40 units /  $\mu$ l.
- Add SialEXO® to the glycoprotein. Add **1 unit SialEXO® / 1  $\mu$ g glycoprotein<sup>3</sup>**.
- Add OpeRATOR® to the glycoprotein. Add **1 unit OpeRATOR® / 1  $\mu$ g glycoprotein<sup>3</sup>**.
- Incubate at **37 °C for 2 h to overnight (16-18 h)**.

#### Option 2

##### Digestion of O-glycosylated protein without removal the sialic acids

- Reconstitute OpeRATOR® in 50  $\mu$ l ddH<sub>2</sub>O<sup>2</sup> to a concentration of 40 units /  $\mu$ l.
- Add OpeRATOR® to the glycoprotein. Add **1 unit OpeRATOR® / 1  $\mu$ g glycoprotein<sup>3</sup>**.
- Incubate at **37 °C overnight (16-18 h)**.

Optimization of enzyme concentrations and incubation time may be needed for a particular protein substrate.

### Notes

1. The OpeRATOR® enzyme has optimal activity in a pH range of pH 5.5 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.

### Legal and Disclaimers

All rights reserved. Genovis products are covered by one or more patents, trademarks and/or copyrights owned or controlled by Genovis AB.

For more information about commercial rights, please contact Genovis team at [info@genovis.com](mailto:info@genovis.com).

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

All goods and services are sold subject to Genovis' General Terms and Conditions of Sale.

©2019 Genovis AB