

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 α2,3-, α2,6- and α2,8-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 ≥ 90% of 1 µg glycoprotein (TNFαR) 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 buffer¹: 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 µl ddH₂O² to a concentration of 40 units / µl.
- Reconstitute OpeRATOR™ in 50 µl ddH₂O² to a concentration of 40 units / µl.
- Add SialEXO™ to the glycoprotein. Add **1 unit SialEXO™ / 1 µg glycoprotein³**.
- Add OpeRATOR™ to the glycoprotein. Add **1 unit OpeRATOR™ / 1 µg glycoprotein³**.
- 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 µl ddH₂O² to a concentration of 40 units / µl.
- Add OpeRATOR™ to the glycoprotein. Add **1 unit OpeRATOR™ / 1 µg glycoprotein³**.
- 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.

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