

INSTRUCTIONS

Version 17.1.2

Instructions for product no:
G2-OG1-020

2000 units

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

Content and Storage

The OglyZOR® box includes:

- 1 vial of OglyZOR® enzyme (G1-OG1-020) 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 OglyZOR® 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. OglyZOR® box is for R&D use only.

Product Description

OglyZOR® is an endoglycosidase that catalyzes the removal of core 1 and 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 α 2-3, α 2-6 or α 2-8 linked sialic acids, is used together with OglyZOR® for efficient removal of the O-linked disaccharides. The SialEXO® is included in the box for convenience.

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 the SialEXO® contain His-tags and the molecular weights are 42.8 kDa and 65.7 kDa, respectively.

Unit Definition

One unit of OglyZOR® removes \geq 90% of O-glycans of 1 μ g glycoprotein (TNFaR) when incubated together with one unit of SialEXO® in 20 mM Tris pH 6.8 at 37 °C for 2 h.

Quality Control

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

Reaction buffer¹: 20 mM Tris buffer pH 6.8

Preparation of glycoprotein

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

Deglycosylation

- Reconstitute SialEXO® in 50 μ l ddH₂O² to a concentration of 40 units / μ l.
- Reconstitute OglyZOR® in 50 μ l ddH₂O² to a concentration of 40 units / μ l.
- Add SialEXO® to the glycoprotein. Add 1 unit SialEXO® / 1 μ g glycoprotein³.
- Add OglyZOR® to the glycoprotein. Add 1 unit OglyZOR® / 1 μ g glycoprotein³.
- Incubate at 37 °C for 2-4 h⁴.

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

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 solutions 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.

4. Longer incubation times may be required depending on the glycoprotein.

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