

## 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 α2-3, α2-6 or α2-8 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 ≥ 90% of O-glycans 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

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 buffer<sup>1</sup>: 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 µl ddH<sub>2</sub>O<sup>2</sup> to a concentration of 40 units / µl.
- Reconstitute OglyZOR™ in 50 µl ddH<sub>2</sub>O<sup>2</sup> to a concentration of 40 units / µl.
- Add SialEXO™ to the glycoprotein. Add **1 unit SialEXO™ / 1 µg glycoprotein<sup>3</sup>**.
- Add OglyZOR™ to the glycoprotein. Add **1 unit OglyZOR™ / 1 µg glycoprotein<sup>3</sup>**.
- Incubate at **37 °C for 2-4 h<sup>4</sup>**.

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.

**OglyZOR™****Limited Use Label License: Research Use Only**

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