

## INSTRUCTIONS

Version 17.1.3

Instructions for product no: G1-SM1-020                      2000 units                      Desialylation of up to 2 mg glycoprotein

### Content and Storage

SialEXO™ is supplied lyophilized in TBS pH 7.6, with no preservatives added.  
SialEXO™ is shipped cold and should be stored at -20 °C upon arrival. After reconstitution SialEXO™ is stable for 1 month at +4-8 °C.  
SialEXO™ is for R&D use only.

### Product Description

SialEXO™ is a mix of sialidases for efficient removal of sialic acids on O-glycosylated and N-glycosylated proteins. The mix is composed of two sialidases for highly efficient hydrolysis of  $\alpha$ 2-3,  $\alpha$ 2-6 or  $\alpha$ 2-8 bonds.  
SialEXO™ hydrolyzes glycoproteins under native conditions and displays a high activity in a broad pH range, 6.5 to 9.

The enzymes in SialEXO™ are derived from *Akkermansia muciniphila* and expressed in *E. coli*. SialEXO™ is composed of two sialidases with His-tags and the molecular weights of the components are 42.8 kDa and 65.7 kDa, respectively.

### Unit Definition

One unit of SialEXO™ hydrolyzes sialic acids from  $\geq$  90% of 1  $\mu$ g glycoprotein (fetuin) when incubated in 20 mM Tris pH 6.8 at 37 °C for 2h.

### Quality Control

SialEXO™ is tested to meet specification.  
SialEXO™ is 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 pH 6.8

#### Preparation of glycoprotein

Prepare the glycoprotein of interest in reaction buffer to a concentration of 0.1-5 mg/ml.

#### 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.
- Add SialEXO™ to the glycoprotein. Add **1 unit SialEXO™ / 1  $\mu$ g glycoprotein<sup>3</sup>**.
- Incubate at **37 °C for 30 min to 2 h<sup>4</sup>**.

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

### Notes

1. SialEXO™ displays high activity in buffers at pH 6.5-9.
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.
4. Longer incubation times may be required depending on the glycoprotein.

**SialEXO™**

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