

## INSTRUCTIONS

Last revised May 2019

Instructions for product no:  
G1-SD2-005

500 units

Desialylation of up to 0.5 mg glycoprotein

### Content and Storage

SialEXO<sup>®</sup> 23 is supplied lyophilized in TBS pH 7.6, with no preservatives added.

SialEXO 23 is shipped cold and should be stored at -20 °C upon arrival. After reconstitution SialEXO 23 is stable for at least 1 month at +4-8 °C.

SialEXO 23 is for R&D use only.

### Product Description

SialEXO 23 is a sialidase for specific removal of  $\alpha$ 2-3 linked sialic acids on O- and N-glycans.

SialEXO 23 hydrolyzes sialic acids on glycans under native conditions and displays a high activity in a broad pH range, 7 to 9.

The enzyme is derived from *Akkermansia muciniphila* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 66 kDa.

SialEXO 23 can be used for exoglycosidase sequencing by combining results from a reaction with the sialidase product SialEXO, that efficiently hydrolyzes  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linked glycosidic bonds.

### Unit Definition

One unit of SialEXO 23 hydrolyzes  $\alpha$ 2-3 linked sialic acids from  $\geq 90\%$  of 1  $\mu$ g glycoprotein (fetuin) when incubated in 20 mM Tris pH 7.5 at 37 °C for 1 h.

### Quality Control

SialEXO 23 is tested to meet specification.

SialEXO 23 is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

SialEXO 23 is tested for activity on 3'-sialyllactose and absence of activity on 6'-sialyllactose.

### Protocol

#### Additional Materials Required

Reaction buffer<sup>1</sup>: 20 mM Tris pH 7.5

#### Preparation of substrate

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

Prepare the oligosaccharide of interest in reaction buffer to a concentration of 1 - 5 pmol/ $\mu$ l.

#### Hydrolysis of $\alpha$ 2-3 linked sialic acids

- Reconstitute SialEXO 23 in 25  $\mu$ l ddH<sub>2</sub>O<sup>2</sup> to a concentration of 20 units /  $\mu$ l.
- Add SialEXO 23 to the reaction. Add **1 unit SialEXO 23 / 1  $\mu$ g glycoprotein or to 5 pmol oligosaccharides<sup>3</sup>**.
- Incubate at **37 °C for 1 h<sup>4</sup>**.

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

#### Notes

1. SialEXO 23 displays high activity in buffers at pH 7-9.
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 or oligosaccharides. This requires optimization.
4. Longer incubation times may be required depending on the substrate.

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