# **GalNAcEXO**<sup>™</sup>

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## **Smart**Enzymes<sup>™</sup>





#### INSTRUCTIONS FOR PRODUCT

GalNAcEXO 2000 units (G1-NA1-020) Digestion of up to 2 mg glycoprotein

Last revised September 2020

## QUICK GUIDE



- Reconstitute GalNAcEXO in 100  $\mu l$  ddH\_20 to a concentration of 20 units/ $\mu l$ 



 Add 1 unit GalNAcEX0 / 1 µg glycoprotein





• Incubate for 2 h to 18 h at 37°C



GalNAcEXO is an exo- $\alpha$ -*N*-Acetylgalactosaminidase for efficient hydrolysis of  $\alpha$ -*N*-acetylgalactosamine (GalNAc) linked to serine or threonine residues in glycoproteins (Tn antigen). GalNAcEXO has some activity on other terminal  $\alpha$ -linked GalNAc. GalNAcEXO hydrolyzes glycoproteins under native conditions and is highly active in a pH range from 6.0 to 7.6. No cofactors or special buffers are required. The enzyme in GalNAcEXO is derived from *Akkermansia muciniphila*, expressed in *E. coli* with a His-tag, and has a molecular weight of 52kDa.

#### **Unit Definition**

One unit of GalNAcEXO catalyzes the hydrolysis of α-linked GalNAc residues from >95% of 2 nmol 4-Nitrophenyl 2-acetamido-2-deoxy-a-Dgalactopyranoside when incubated in 20 mM Tris, 1% EtOH pH 6.8 at 37°C for 30 min.



#### **Content and Storage**

GalNAcEXO is supplied lyophilized in TBS pH 7.6, with no preservatives added.

GalNAcEXO is shipped cold and should be stored at -20°C upon arrival.

After reconstitution GalNAcEXO is stable for 1 month at +4-8°C.

GalNAcEXO is for R&D use only.

### DETAILED PROTOCOL

#### **Additional Materials Required**

Reaction buffer<sup>1</sup>: 20 mM Tris pH 6.8

#### Sample Preparation

• Prepare the glycoprotein of interest in the reaction buffer at a concentration of 0.5-5.0 mg/ml.

#### **Digestion of Glycoproteins**

#### Prepare GalNAcEXO

 Reconstitute GalNAcEXO in 100µl ddH<sub>2</sub>O to a concentration of 20 units/µl

#### 2 Add GalNAcEXO

Add 1 unit GalNAcEXO / 1µg glycoprotein<sup>2</sup>

#### **3** Digestion

Incubate for 2 to 18h at 37°C

Optimization of enzyme concentrations and incubation time may be needed depending on the substrate.

#### Notes

- GalNAcEXO displays high activity in buffers with pH values from 6.0 to 7.6 and over a wide range of ionic strength. Some optimizations might be required if a buffer other than the recommended reaction buffer is used.
- A higher enzyme concentration may increase digestion efficiency of individual glycoproteins. This requires optimization.

#### **Quality Control**

GalNAcEXO is tested to meet the specifications and lot-to-lot consistency.

GalNAcEXO is tested for the absence of microbial contamination using blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

#### **Related Products**

#### SialEXO<sup>®</sup>

For complete removal of  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linked sialic acids.

#### GalactEXO™

GalNAcEXO for complete removal of both  $\beta$ 1-3 and  $\beta$ 1-4 linked galactoses.

#### **OglyZOR®**

Specific hydrolysis of core 1 O-glycan disaccharides on native glycoproteins.

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