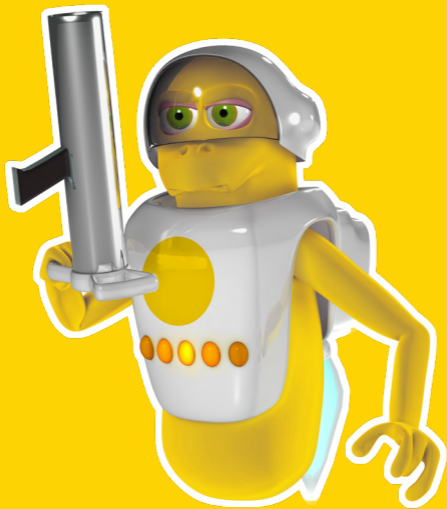


# GalactEXO™

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## SmartEnzymes™

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## INSTRUCTIONS FOR PRODUCT

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**GalactEXO 2000 units** (G1-GM1-020)

Digestion of up to 2 mg glycoprotein

## 1 Prepare GalactEXO

- Reconstitute GalactEXO in 100  $\mu$ l ddH<sub>2</sub>O to a concentration of 20 units/ $\mu$ l



## 2 Add GalactEXO

- Add 1 unit GalactEXO / 1  $\mu$ g glycoprotein or 1 pmol oligosaccharides



## 3 Digestion

- Incubate for 2 h to 18 h at 37°C



# PRODUCT DESCRIPTION

GalactEXO is a mix of  $\beta$ -galactosidases for efficient removal of galactose residues on N- and O-glycosylated proteins or oligosaccharides. The mix is composed of two galactosidases for highly efficient hydrolysis of both  $\beta$ 1-3 and  $\beta$ 1-4 linked galactoses<sup>1</sup>. GalactEXO hydrolyzes glycoproteins under native conditions and displays a high activity in a broad pH range, 5.5 to 7.5. The enzymes in GalactEXO are derived from *Akkermansia muciniphila* and expressed in *E. coli*. The two galactosidases have his tags and the molecular weights of the components are 87 kDa and 109 kDa, respectively.

## Unit Definition

One unit of GalactEXO hydrolyzes galactoses from >95 % of 0.15 nmol Gal- $\beta$ 1,3-GalNAc-pNP and 2 nmol Gal- $\beta$ 1,4-GlcNAc-pNP when incubated in 20 mM Tris pH 6.8 at 37°C for 30 min.

## Content and Storage

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

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

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

GalactEXO is for R&D use only.

# DETAILED PROTOCOL

## Additional Materials Required

- Reaction buffer<sup>2</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

### 1 Prepare GalactEXO

- Reconstitute GalactEXO in 100  $\mu$ l ddH<sub>2</sub>O to a concentration of 20 units/ $\mu$ l

### 2 Add GalactEXO

- Add 1 unit GalactEXO / 1  $\mu$ g glycoprotein<sup>3</sup>

### 3 Digestion

- Incubate for 2 to 18 h at 37°C

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

## Digestion of Oligosaccharides

### 1 Prepare GalactEXO

- Reconstitute GalactEXO in 100  $\mu$ l ddH<sub>2</sub>O to a concentration of 20 units/ $\mu$ l

### 2 Add GalactEXO

- Add 1 unit GalactEXO / 1 pmol oligosaccharides

### 3 Digestion

- Incubate for 1 h at 37°C

#### Notes

1. GalactEXO also hydrolyses  $\beta$ 1-6 linked galactoses to a certain extent.
2. GalactEXO displays high activity in buffers with pH values from 5.5 to 7.5 and over a wide range of ionic strength. Some optimizations might be required if a buffer other than the recommended reaction buffer is used.
3. A higher enzyme concentration may increase digestion efficiency of individual glycoproteins. This requires optimization.

## Quality Control

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

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

## Related Products

### **SialEXO®**

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

### **SialEXO® 23**

For complete and specific removal of  $\alpha$ 2-3 linked sialic acids.

### **Immobilized SialEXO®**

Complete removal of sialic acids in a spin column format.



## Legal and Disclaimers

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## SialEXO<sup>®</sup>

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Efficient removal of sialic acids on O-glycosylated and N-glycosylated proteins

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.



# SialEXO<sup>®</sup> 23

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Specific removal of  $\alpha$ 2-3 linked sialic acids on O- and N-glycans

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.





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