Immobilized

GalactEXO™

FOR RESEARCH
USE ONLY

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STORE AT
+4-8°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

Immobilized GalactEXO™ Microspin 2×0.5mg
Digestion of up to 2×0.5mg glycoprotein (G1-GM6-010)

Immobilized GalactEXO™ Microspin 5×0.5mg
Digestion of up to 5×0.5mg glycoprotein (G1-GM6-025)

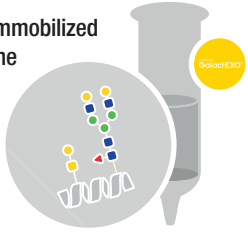
Immobilized GalactEXO™ Microspin 10×0.5mg
Digestion of up to 10×0.5mg glycoprotein (G1-GM6-050)

Last revised September 2020

1 Equilibration

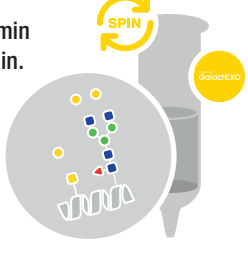
- Equilibrate the column with 3x300µl digestion buffer. Centrifuge at 200xg for 1 min.

2 Digestion



- Add the glycoprotein to the Immobilized GalactEXO column and cap the column. Incubate at room temperature with end-over-end mixing for 30-60 min.

3 Collection



- Centrifuge at 1000xg for 1 min to collect the digested protein.
- For maximum recovery, add 100µl reaction buffer, invert and centrifuge at 1000xg for 1 min.
- Repeat once.

Immobilized GalactEXO is a resin with a mixture of two of β-galactosidases covalently coupled to agarose beads for efficient removal of galactose residues (β1-3 and β1-4 linked[1]) on N- and O-glycosylated proteins. The enzymes in GalactEXO are derived from *Akkermansia muciniphila* and expressed in *E. coli*. Degalactosylated proteins are generated without the enzymes in the final preparation. The glycoprotein sample is incubated with the Immobilized GalactEXO resin and the digested glycoproteins are then easily collected by a centrifugation step. The recommended buffer for Immobilized GalactEXO is 20 mM Tris pH 6.8[2,3]. The protocol may need optimization regarding buffer compatibility and incubation time for individual glycoproteins.

Content and Storage

Immobilized GalactEXO Microspin columns contain sufficient material each to remove galactoses from 0.5 mg glycoprotein. The resin is supplied in 20 % EtOH with no preservatives added.

Immobilized GalactEXO is shipped cold and should be stored at +4-8 °C upon arrival.
Do not freeze the product!

Immobilized GalactEXO is for R&D use only.

Use lids and bottom caps during the incubation.

Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).

Additional Materials Required

- Reaction buffer[2]: 20 mM Tris pH 6.8
- Collection tubes: Microcentrifuge tubes (1.5-2 ml)

Sample Preparation

- Prepare the glycoprotein in 100-300 µl reaction buffer per column. Max amount of glycoprotein is 0.5 mg per column.

Digestion of Glycoprotein on Immobilized GalactEXO Column

1 Equilibration

- Break off the bottom cap of the column (save the cap) and place the column in a collection tube. Loosen the lid.
- Centrifuge at 200 × g for 1 min to remove the storage solution.
- Equilibrate the column by adding 300 µl reaction buffer and centrifuge at 200 × g for 1 min.
- Repeat the equilibration step two times.
- Seal the spin column with the bottom cap.

2 Digestion

- Add the glycoprotein to be digested in a volume of 100-300 µl digestion buffer. Max 0.5 mg glycoprotein per column.
- Seal the column with the top lid.
- Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- Incubate the column with end-over-end mixing at room temperature for 30-60 min[4].

3 Collection of Digested Protein

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1000 × g for 1 min to recover the digested glycoproteins.
- For Maximum Recovery of the Sample:
  - Seal the spin column with the bottom cap.
  - Add 100 µl reaction buffer[3].
  - Seal the column and invert the column a couple of times.
  - Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at 1000 × g for 1 min to collect the material.
- Repeat once.
- Pool the collected fractions.

Notes

1. Immobilized GalactEXO also hydrolyses β1-6 linked galactoses to a certain extent.
2. Immobilized 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. If the glycoprotein sticks to the resin, a buffer with higher salt concentration can be used in the reaction and/or in the wash steps.
4. Longer incubation times may be required depending on the glycoprotein.

Quality Control

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

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

Related Products

GalactEXO™
For complete removal of β1-3 and β1-4 linked galactoses.

SialEXO®
For complete removal of α2-3, α2-6 and α2-8 linked sialic acids.

Immobilized SialEXO®
Complete removal of sialic acids in a spin column format.

Legal and Disclaimers

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