Immobilized

FucosEXO™

FOR RESEARCH
USE ONLY

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STORE AT
+4-8°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

Immobilized FucosEXO™ Microspin 5 columns
Digestion of up to 5 × 0.5 mg glycoprotein (G1-FM6-025)

Immobilized FucosEXO™ Microspin 10 columns
Digestion of up to 10 × 0.5 mg glycoprotein (G1-FM6-050)

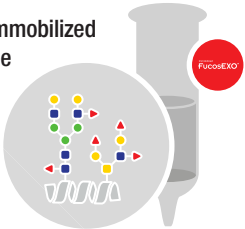
Last revised Mar 2021

1 Equilibration

- Equilibrate the column with 3 x 300 µl digestion buffer. Centrifuge at 200 x g for 1 min.

2 Digestion

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



3 Collection

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

PRODUCT DESCRIPTION

Immobilized FucosEXO is a resin with a mixture of two α-fucosidases covalently coupled to agarose beads for efficient removal of of α1-2, α1-3 and α1-4-linked fucose residues on *N*- and *O*-glycosylated proteins. The enzymes in Immobilized FucosEXO are expressed in *E. coli*. Defucosylated proteins are generated without enzymes in the final preparation. The glycoprotein sample is incubated with the Immobilized FucosEXO resin and the digested glycoproteins are then easily collected by a centrifugation step. The recommended buffer for Immobilized FucosEXO is 20 mM Tris pH 6.8[1,2]. The protocol may need optimization regarding buffer compatibility and incubation time for individual glycoproteins.

Content and Storage

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

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

Immobilized FucosEXO Microspin 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[1]: 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 FucosEXO 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 reaction 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 60 min[3].

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[2].
  - 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 FucosEXO displays high activity in buffers with pH values from 6 to 8 and over a wide range of ionic strength (0-500 mM NaCl). Some optimizations might be required if a buffer other than the recommended reaction buffer is used.
2. 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.
3. Longer incubation times may be required depending on the glycoprotein.

Quality Control

Immobilized FucosEXO 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.

Immobilized GalactEXO™
Complete removal of β1-3 and β1-4 linked galactoses in a spin column format.

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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