



GaINAcEXO™

Immobilized

STORE AT

+4-8°C



FOR RESEARCH USE ONLY

Instructions for Use

GaINAcEXO™ Immobilized

Microspin 5 × 0.5 mg (G1-NA6-025)

Process 5 × 0.5 mg glycoprotein

GaINAcEXO™ Immobilized

Microspin 10 × 0.5 mg (G1-NA6-050)

Process 10 × 0.5 mg glycoprotein



Preparations

Important Information

- 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: 20mM Tris pH 6.8.^{1,2}
- Microcentrifuge tubes (1.5-2 ml)

1. GalNAcEXO Immobilized displays high activity in buffers with pH 6.0-7.6 and over a wide range of ionic strengths (0-500mM NaCl). Optimization 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.

Hydrolysis of α -linked GalNAcs in Spin Columns

Sample Preparation

Prepare the glycoprotein in 100-300 μ l reaction buffer per column. Use 0.5 mg of glycoprotein per column.

1. Equilibration

- 1.1 Break off the bottom cap of the column (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.
- 1.2 Centrifuge at 200 \times g for 1 min to remove the storage solution. Discard the flow-through.
- 1.3 Equilibrate the column by adding 300 μ l reaction buffer and centrifuge at 200 \times g for 1 min. Discard the flow-through.
- 1.4 Perform step 1.3 two additional times.
- 1.5 Insert the bottom cap.

2. Enzymatic Reaction

- 2.1 Add the glycoprotein in a volume of 100-300 μ l reaction buffer. Use 0.5 mg glycoprotein per column.
- 2.2 Seal the column with the lid.
- 2.3 Fully suspend the media, mix by inversion and make sure there is a flow in the column.
- 2.4 Incubate the column with end-over-end mixing at room temperature for 2-4 hours³.

3. Collection of Processed Material

- 3.1 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
- 3.2 Centrifuge at 1000 \times g for 1 min to collect the processed material.

4. For Maximum Recovery of the Sample:

- 4.1 Insert the bottom cap.
- 4.2 Add 100 μ l reaction buffer.²
- 4.3 Seal the column with the lid and invert it a couple of times.
- 4.4 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
- 4.5 Centrifuge at 1000 \times g for 1 min to collect the processed material.
- 4.6 Repeat steps 4.1-4.5.
- 4.7 Pool the collected fractions, including the sample from step 3.2.

3. Longer incubation times may be required depending on the glycoprotein.

USA & Canada

Genovis Inc.

245 First Street, Suite 1800, Cambridge, MA 02142, USA

Phone: 1-855-782-0084 (toll free)

Fax: 1-858-524-3006

EMEA & Asia

Genovis AB

Box 4, SE-24421 Kävlinge, Sweden

Phone: +46 46 10 12 30

Fax: +46 46 12 80 20

support@genovis.com

www.genovis.com



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