



GlycOCATCH®

Affinity Purification

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Instructions for Use

GlycOCATCH® Affinity Purification

Microspin 0.2mg (G3-OC6-002)

Enrich 0.2 mg O-glycoprotein

DOWNLOAD INSTRUCTIONS FOR USE



www.genovis.com/ifu-G3-OC6

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).
- If the sample has been treated with proteases (like trypsin), relevant inhibitors must be added before applying the sample to the column.

Additional Materials Required

- Binding buffer: PBS (10-150 mM sodium phosphate, 150 mM NaCl, pH 7.4) or 50-150 mM Tris, 150 mM NaCl, pH 7.0-8.0.¹
- Wash buffer: Binding buffer with optional addition of up to 1 M NaCl and/or 0.5 M urea.
- **Elution buffer option A – with urea:** 8 M urea in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4).
Elution buffer option B – with OpeRATOR: 50 units of OpeRATOR in 100 µl PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4) for elution of one column.
- Microcentrifuge tubes: 1.5-2 ml.

1. Optimal binding is obtained in sodium phosphate (10-150 mM) and Tris (50-150 mM) buffers at pH 6.0-8.0. NaCl up to 1 M and/or 0.5 M urea can be added without affecting the performance of the column/purification. Optimal buffer conditions may need to be tested for the glycoprotein solution to be purified.

Enrichment of O-glycopeptides

Sample Preparation

Prepare the protein sample solution in 100-300 μ l binding buffer/column.² The sample solution can contain up to 0.5 M urea.

1. Reconstitution of Lyophilized Enzymes

- 1.1 Reconstitute SialEXO in 20 μ l ddH₂O to a concentration of 10 units/ μ l.
- 1.2 If elution will be performed with OpeRATOR (see elution Option B): reconstitute OpeRATOR in 20 μ l ddH₂O to a concentration of 10 units/ μ l.

2. Equilibration

- 2.1 Break off the bottom cap of the GlycOCATCH Microspin column³ (save the cap), and place the column in a microcentrifuge tube. Loosen the lid.
- 2.2 Centrifuge at 200 \times g for 1 min to remove the storage solution. Discard the flow-through.
- 2.3 Equilibrate the column by adding 300 μ l binding buffer and centrifuge at 200 \times g for 1 min. Discard the flow-through.
- 2.4 Perform step 2.3 two additional times.
- 2.5 Insert the bottom cap.

2. The volume should be at least 100 μ l/column and can be increased up to 300 μ l/column.
3. Four GlycOCATCH Affinity Purification Microspin columns are included. Each column contains sufficient material to bind 50 μ g O-glycoprotein.

3. Desialylation and Binding

- 3.1 Add 50 units of SialEXO to the prepared sample solution (100-300 μ l) and add the solution to the column.⁴
- 3.2 Seal the column with the lid.
- 3.3 Fully suspend the media, mix it by inversion and make sure there is a flow in the column.
- 3.4 Incubate the column with end-over-end mixing at room temperature for 30 min-2 h.⁵ A good mixing is important for optimal performance.
- 3.5 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
- 3.6 Centrifuge at 1000 \times g for 1 min to collect the flow-through.
- 3.7 Insert the bottom cap.

4. Washing

- 4.1 Wash the column by adding 300 μ l wash buffer. Seal the column with the lid. Mix by inversion.
- 4.2 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
- 4.3 Centrifuge at 200 \times g for 1 min.
- 4.4 Insert the bottom cap.
- 4.5 Perform steps 4.1-4.4 at least three to five additional times.⁶

4. Sialic acids need to be removed for optimal performance. Optionally, desialylation of glycoproteins with SialEXO can be performed before affinity purification on the GlycOCATCH column by adding 1 unit of SialEXO/ μ g glycoprotein and incubate at 37°C for 2h.
5. Incubation time may need optimization depending on sample composition and glycoproteins.
6. Extended washing, up to ten or more times, may decrease unspecific binding.

5. Elution of Bound Material

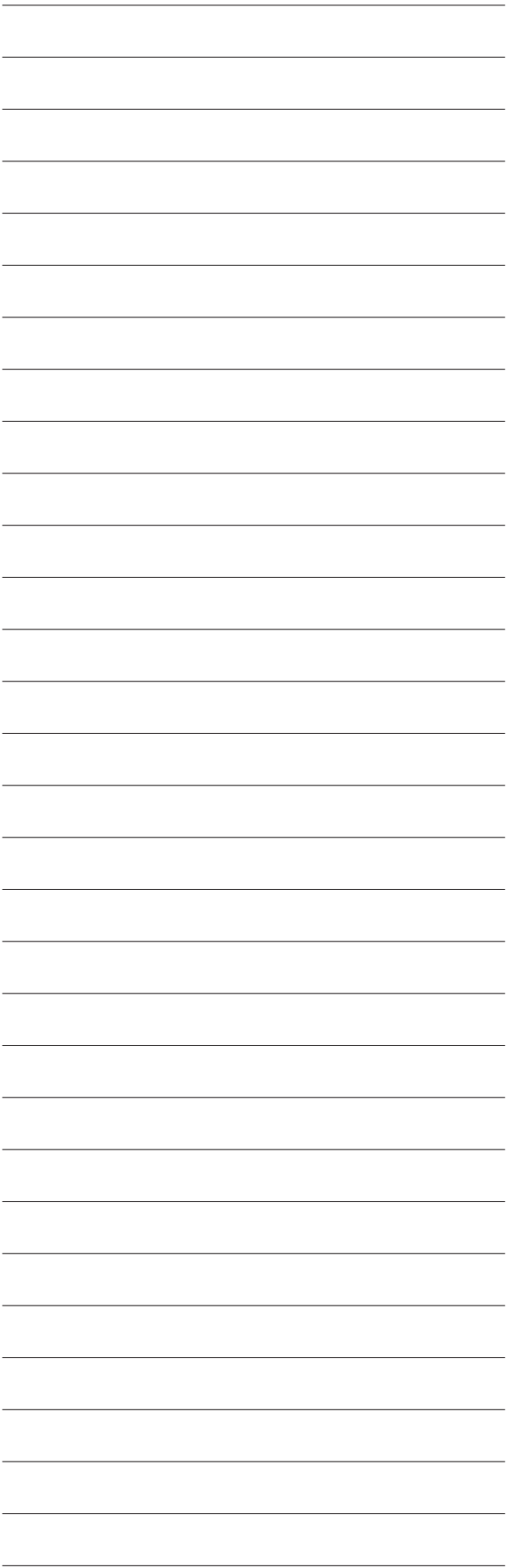
Option A – with Urea

- 5.A1 Add 50 µl Elution buffer Option A to the column.
- 5.A2 Seal the column with the lid and mix by tapping the tube and incubate for 5 min at RT.
- 5.A3 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
- 5.A4 Centrifuge at 1000×g for 1 min to collect the eluted bound material.
- 5.A5 Insert the bottom cap and repeat steps 5.A1-5.A4. Use the same microcentrifuge tube.

Option B – with OpeRATOR

- 5.B1 Add 100 µl Elution buffer Option B (50 units OpeRATOR) to the column.
- 5.B2 Seal the column with the lid and fully suspend the media, mix by inversion and make sure there is a flow in the column.
- 5.B3 Incubate the column with end-over-end mixing overnight at 37°C. A good mixing is important for optimal performance.
- 5.B4 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
- 5.B5 Centrifuge at 1000×g for 1 min to collect the eluted bound material.⁷

- 7. After elution of the O-glycan-containing fraction, the samples can be further processed with trypsin (or other suitable protease) and prepared for LC-MS analysis. After elution with OpeRATOR, long peptides can be generated depending on the position of the O-glycans and the distance between O-glycan sites. Further proteolytic digestion with trypsin (or other suitable protease) may be needed to obtain shorter peptides prior to LC-MS analysis.



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