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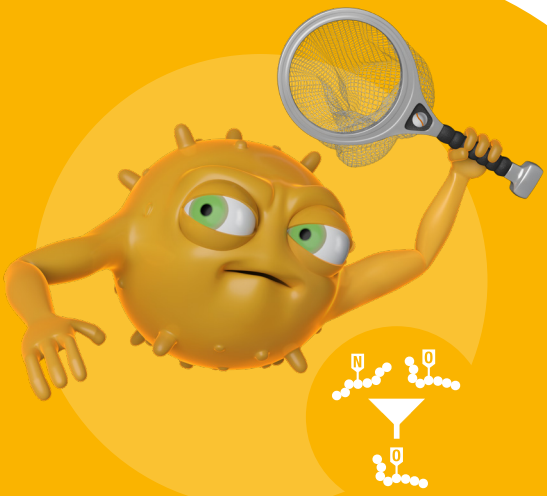

GlycOCATCH™

Affinity Purification

STORE AT
DIFFERENT
TEMPERATURES

FOR RESEARCH USE ONLY

Instructions for Use

GlycOCATCH™ Affinity Purification
Microspin 0.2 mg (G3-OC6-002)
Enrich 0.2 mg O-glycoprotein

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-G3-OC6

Affinity Resin for Enrichment of O-glycopeptides

GlycOCATCH is an affinity resin for purification of mucin-type O-glycosylated proteins and peptides. The affinity resin is based on inactive OpeRATOR® enzyme that has been engineered to bind O-glycosylated proteins and peptides with high affinity.

GlycOCATCH Affinity Purification consists of microspin columns with GlycOCATCH resin for binding of mucin-type O-glycosylated proteins and peptides, SialEXO® Lyophilized for removal of sialic acids and OpeRATOR Lyophilized for an optional elution method of the bound O-glycoprotein. When using this elution method, OpeRATOR digests the bound O-glycoproteins N-terminally of the O-glycan sites, and peptides are recovered under native conditions. Elution can also be performed using 8 M urea. Since asialylated O-glycans bind more efficiently to the GlycOCATCH resin, the sialidase mix SialEXO is included.

The SialEXO and OpeRATOR enzymes are derived from *Akkermansia muciniphila* and expressed in *E. coli*. The SialEXO enzymes contain His-tags and have molecular weights of 43 and 66 kDa. The OpeRATOR enzyme contains a His-tag and has a molecular weight of 42 kDa.

CONTENT AND STORAGE

GlycOCATCH Affinity Purification contains three components. The product box is shipped cold, and the components should be stored at different temperatures upon arrival.

- **4 GlycOCATCH Affinity Purification Microspin columns** are supplied in 20% ethanol, with no preservatives added. One column contains sufficient material to bind 50 µg O-glycoprotein. The columns should be stored at +4-8°C upon arrival. **Do not freeze the columns!**
- **1 vial SialEXO Lyophilized** (200 units) supplied in TBS pH 7.6, with no preservatives added. SialEXO Lyophilized should be stored at -20°C upon arrival. After reconstitution, SialEXO Lyophilized is stable for at least 1 month at +4-8°C.
- **1 vial OpeRATOR Lyophilized** (200 units) supplied in TBS pH 7.6, with no preservatives added. OpeRATOR Lyophilized should be stored at -20°C upon arrival. After reconstitution, OpeRATOR Lyophilized is stable for at least 1 month at +4-8°C.

GlycOCATCH Affinity Purification is for R&D use only.

BINDING CAPACITY AND UNIT DEFINITIONS

- 50 µl GlycOCATCH Affinity Purification resin binds ≥ 50 µg asialylated etanercept when incubated in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4 at room temperature for 30 minutes.
- One unit SialEXO Lyophilized hydrolyzes sialic acids from ≥ 90% 1 µg glycoprotein (fetuin) when incubated in 20 mM Tris, pH 6.8 at 37°C for 2 hours.
- One unit OpeRATOR Lyophilized digests ≥ 90% of 1 µg glycoprotein (TNFR) when incubated together with one unit SialEXO Lyophilized in 20 mM Tris, pH 6.8 at 37°C for 2 hours.

QUALITY CONTROL

All components in GlycOCATCH Affinity Purification are tested to meet the specifications and lot-to-lot consistency. GlycOCATCH Affinity Purification is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

YOU MIGHT ALSO BE INTERESTED IN

OpeRATOR™
O-glycan-specific protein digestion

ImpaRATOR™
O-glycan-specific protein digestion, including sialylated O-glycan species

OglyZOR™ Lyophilized
Lyophilized enzyme for hydrolysis of core 1 O-glycans

SialEXO™ Immobilized
Immobilized enzyme mix for hydrolysis of sialic acids in spin columns

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 µl 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 µl ddH_2O to a concentration of 10 units/µl.

1.2 If elution will be performed with OpeRATOR (see elution Option B): reconstitute OpeRATOR in 20 µl ddH_2O to a concentration of 10 units/µl.

2. Equilibration

2.1 Break off the bottom cap of the GlycOCATCH Microspin column[3] (save the cap), and place the column in a microcentrifuge tube. Loosen the lid.

2.2 Centrifuge at 200 × g for 1 min to remove the storage solution. Discard the flow-through.

2.3 Equilibrate the column by adding 300 µl binding buffer and centrifuge at 200 × 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 µl/column and can be increased up to 300 µl/column.
3. Four GlycOCATCH Affinity Purification Microspin columns are included. Each column contains sufficient material to bind 50 µg O-glycoprotein.

3. Desialylation and Binding

3.1 Add 50 units of SialEXO to the prepared sample solution (100-300 µl) 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 × 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 µl 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 × 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/µg glycoprotein and incubate at 37°C for 2 h.
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.

USA & Canada
Genovis Inc.
10919 Technology Place Suite C, San Diego, CA 92127, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 4, SE-244 21 Kävlinge, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

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