# GlycOCATCH™

GENOVIS

## INSTRUCTIONS

**Last revised April 2018**

Instructions for product no:

G3-OC6-002 Binding of up to 4 × 50 µg O-glycoprotein

## Content and storage

GlycOCATCH™ box contains:

- 4 Microspin columns with GlycOCATCH™ resin (G1-OC6-001), each column includes sufficient material to bind 50 µg O-glycoprotein. The resin is supplied in 20% EtOH with no preservatives added.
- 1 vial of SialEXO™ (200 units, G1-SM1-002) supplied lyophilized in TBS pH 7.6, with no preservatives added.
- 1 vial of OpeRATOR™ (200 units, G1-OP1-002) supplied lyophilized in TBS pH 7.6, with no preservatives added.

GlycOCATCH™ box is shipped cold.
GlycOCATCH™ Microspin columns should be stored at +4-8°C upon arrival. **Do not freeze the columns!**
SialEXO™ and OpeRATOR™ should be stored at -20°C upon arrival. After reconstitution, the SialEXO™ and OpeRATOR™ are stable for 1 month at +4-8 °C.

GlycOCATCH™ box is for R&D use only.

## Product Description

GlycOCATCH™ is a resin for affinity purification and selective enrichment of O-glycoproteins and O-glycopeptides. The affinity ligand is an engineered inactive version of the O-glycan dependent OpeRATOR™ protease. The GlycOCATCH™ agarose resin specifically binds O-glycoproteins and O-glycopeptides, selectively targeting O-glycan and not N-glycan sites. Asialylated O-glycans on O-glycoproteins are bound efficiently to the resin and the sialidase mix SialEXO™, for removal of sialic acids, is included in the box.

In general, O-glycoproteins and O-glycopeptides bind to the resin at pH 5-8 and are recovered with 8 M urea (i.e. in a denatured state) in a 1 hour workflow. Alternatively, the OpeRATOR™ enzyme, an O-protease, can be used as an elution method. Bound O-glycoproteins are then eluted as peptides, with a size dependent on the positions of the O-glycan sites. At elution, the bound O-glycoproteins are digested N-terminally of the O-glycan sites, without the presence of a denaturing agent, and resulting peptides are recovered under native conditions.

SialEXO™ is a mix of sialidases for efficient removal of sialic acids on O-glycosylated and N-glycosylated proteins. SialEXO™ hydrolyzes glycoproteins under native conditions and displays a high activity in a broad pH range, 6.5 to 9. The mix is composed of two sialidases for highly efficient hydrolysis of α2-3, α2-6 or α2,8-linked sialic acids. The enzymes in SialEXO™ are derived from *A. muciniphila* and expressed in *E. coli*. The enzymes contain His-tags and the molecular weights are 42.8 kDa and 65.7 kDa, respectively.

OpeRATOR™ enzyme is an endoprotease digesting O-glycosylated proteins N-terminally of the Serine and Threonine glycosylation sites. The OpeRATOR™ is an O-protease which is active on glycoproteins under native conditions. It is highly specific and digests the peptide backbone only in presence of O-glycans, generating distinct O-glycopeptides suitable for applications such as O-glycan profiling, O-glycan site determination, O-glycopeptide mapping and middle down approaches. OpeRATOR™ is active on O-glycan proteins with sialic acids but the enzymatic activity is higher if the sialic acids are removed. OpeRATOR™ enzyme is derived from *A. muciniphila* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 41.8 kDa.

## Binding Capacity and Unit Definitions

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

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## Quality Control

All components in the GlycOCATCH™ box are tested to meet specifications and tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

## Additional Materials Required

- Binding buffer[1]: PBS (10-150 mM sodium phosphate, 150 mM NaCl, pH 7.4) or 50-150 mM Tris, 150 mM NaCl, pH 7-8.
- Wash buffer: Binding buffer with optional addition of up to 1 M NaCl and/or 0.5 M urea.
- Elution buffer for elution with urea: 8 M urea in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4).
- Elution buffer for elution 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.
- Collection tubes: Micro centrifuge tubes (1.5-2 ml).

## Detailed Protocol

- Lids and bottom caps are used during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).

### Sample preparation

- Prepare the protein sample solution to be affinity purified in 100-300 µl binding buffer[2] / column. The sample solution can contain up to 0.5 M urea.

  **Please note**: If the sample has been treated with proteases (like trypsin) relevant inhibitors must be added before applying the sample on the column.

### Affinity Purification on the GlycOCATCH™ column

- Reconstitute SialEXO™ in 20 µl $ddH_2O$ to a concentration of 10 units / µl.
- If elution will be performed with OpeRATOR™ (see below Elution Option 2): reconstitute OpeRATOR™ in 20 µl $ddH_2O$ to a concentration of 10 units / µl.

#### *Equilibration*

1. Break off the bottom seal (save the cap) of the column and loosen the lid.
2. Place the column in a 1.5-2 ml collection tube.
3. Centrifuge the column at 200×g for 1 min to remove the storage solution.
4. Equilibrate the column by adding 300 µl binding buffer.
5. Centrifuge the column at 200×g for 1 min.
6. Repeat steps 4 and 5 two times.
7. Seal the spin column with the bottom cap.

#### *Desialylation and Binding*

8. Add 50 units of SialEXO™ to the O-glycoprotein sample solution for one column[3].
9. Add the prepared protein sample solution (100-300 µl) to the column.
10. Seal the column with the top lid.
11. Take care to fully suspend the media, mix by inversion and **make sure the resin is flowing in the column**.
12. Incubate the column by end-over-end mixing at room temperature for 30 min - 2 h[4]. **A good mixing is important for optimal performance.**
13. Remove the bottom cap and place the column in a 1.5-2 ml collection tube. Loosen the top lid.
14. Centrifuge the column at 1000xg for 1 min to collect the flow through (FT).
15. Seal the spin column with the bottom cap.

***Washing***

16. Wash the column by adding 300 µl wash buffer. Mix by inversion of the column.
17. Remove the bottom cap and place the column in a 1.5-2 ml collection tube. Loosen the top lid.
18. Centrifuge the column at 200×g for 1 min.
19. Seal the spin column with the bottom cap.
20. Repeat washing steps 16-19 at least 3-5 times[5].
21. Seal the spin column with the bottom cap.

***Elution of O-glycoproteins / O-glycopeptides***

***Option 1- with urea***

1. Add 50 µl elution buffer (8 M urea in PBS) to the column.
2. Seal the column and mix by tapping the tube and incubate at RT for 5 min.
3. Remove the bottom cap and place the column in a 1.5-2 ml collection tube. Loosen the top lid.
4. Centrifuge the column at 1000×g for 1 min to collect the eluate.
5. Repeat elution steps 1 to 4 once more. Use the same collection tube.

***Option 2 – with OpeRATOR***

1. Add 50 units of OpeRATOR™ in 100 µl PBS for one column.
2. Take care to fully suspend the media, mix by inversion and **make sure the resin is flowing in the column**.
3. Incubate the column by end-over-end mixing at 37°C overnight. **A good mixing is important for optimal performance.**
4. Remove the bottom cap and place the column in a 1.5-2 ml collection tube. Loosen the top lid.
5. Centrifuge the column at 1000×g for 1 min to collect the eluate.

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.

***Notes***

1. Optimal binding is obtained in sodium phosphate (10-150 mM) and Tris (50-150 mM) buffers at pH 6.0-8.0. Sodium chloride 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.
2. The volume should be at least 100 µl / column and can be increased up to 300 µl / column.
3. Sialic acids need to be removed for optimal performance of GlycOCATCH™. Optionally, desialylation of glycoproteins with SialEXO™ can be performed before affinity purification on the GlycOCATCH™ column by adding 1 unit of SialEXO™ / µg of glycoprotein and incubate at 37°C for 2 h.
4. Incubation time may need optimization depending on sample composition and glycoproteins.
5. Extended washing, up to ten or more times, may decrease unspecific binding.

**GlycOCATCH™**
**Limited Use Label License: Research Use Only**

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