Immobilized GlycINATOR®

GENOVIS

INSTRUCTIONS

Version 17.1.1

Intructions for product no

A0-GL6-010	2 columns	Deglycosylation of up to 2 × 0.5 mg IgG
A0-GL6-025	5 columns	Deglycosylation of up to 5 × 0.5 mg IgG
A0-GL6-050	10 columns	Deglycosylation of up to 10 × 0.5 mg IgG

Product Description

GlycINATOR® (EndoS2) is an endoglycosidase for deglycosylation of the Fc N-glycan moieties of IgGs (1). All IgG glycoforms are hydrolyzed, including high mannose, hybrid type and bisected glycans (2). GlycINATOR® hydrolyzes the β1,4 linkage between the core GlcNAc residues in the Fc-glycan, leaving the innermost GlcNAc on the Fc. GlycINATOR® deglycosylates all human IgG subclasses and IgG from the following species: mouse, rat, monkey, sheep, goat, cow and horse. It has also been reported to hydrolyze glycan moieties from alpha-1-acid glycoprotein.

The Immobilized GlycINATOR® Microspin columns contains GlycINATOR® covalently coupled to agarose beads for deglycosylation of Fc-glycans. IgG is incubated with the GlycINATOR® agarose beads for 15 min, deglycosylated IgG is then collected by a 1 minute centrifugation step.

Content and storage

Spin columns containing GlycINATOR® covalently coupled to agarose beads.

The GlycINATOR® Microspin columns are supplied in 20% EtOH and no preservatives are added.

One microspin column contains sufficient GlycINATOR® coupled agarose beads to deglycosylate 0.5 mg IgG.

GlycINATOR® Microspin columns are shipped on ice. GlycINATOR® Microspin columns should be stored at +4-8°C upon arrival.

The GlycINATOR® Microspin columns are for R&D use only.

Additional Materials Required

- Reaction buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Collection tubes: Micro centrifuge tubes.

Method

- Make sure your antibody is in reaction buffer (See Additional Materials Required above). Prepare a maximum of 0.5 mg IgG in 100-300 µl reaction buffer.
- Lids and bottom caps are used during the incubation.
- Before centrifugation remove the bottom cap and loosen the lid (do not remove it). Remember to save the bottom cap!

1. Break off the bottom cap of the spin column (save the cap) and loosen the lid.
2. Centrifuge the column at 200×g for 1 min to remove storage solution.
3. Equilibrate the column with 300 µl reaction buffer (See Additional Materials Required above).
4. Centrifuge the column at 200×g for 1 min.
5. Repeat steps 3 and 4 two times.
6. Re-insert the bottom cap into the bottom of the spin column.
7. Immediately add 100-300 µl IgG, maximum 0.5 mg IgG, in reaction buffer.
8. Re-seal the column with the lid.
9. Take care to fully suspend the media manually and make sure it is flowing in the column.

10. Incubate the column by end-over-end mixing at room temperature for 15 min. The incubation time can be increased if necessary.
11. Twist open the lid and remove the bottom cap.
12. Place the column in a clean micro centrifuge tube (not included).
13. Centrifuge the column at 1000×g for 1 min to elute the sample.

For maximum recovery of the sample:

14. Place the column in a clean micro centrifuge tube (not included).
15. Add 100 µl reaction buffer to the column.
16. Centrifuge the column at 1000×g for 1 min to elute the sample.
17. Repeat steps 14-16 one more time.

1. Sjögren, J. et al., 2013. EndoS2 is a unique and conserved enzyme of serotype M49 group A Streptococcus that hydrolyses N-linked glycans on IgG and α1-acid glycoprotein. The Biochemical Journal, 455(1), pp.107–118.
2. Sjögren, J. et al., 2015. EndoS and EndoS2 hydrolyze Fc-glycans on therapeutic antibodies with different glycoform selectivity and can be used for rapid quantification of high-mannose glycans. Glycobiology, 25(10), pp.1053–1063.