

INSTRUCTIONS

Version 17.1.1

Instructions for product no:

A0-GL6-100 1 column, Midispin Deglycosylation of up to 10 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 β 1,4 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® Midispin columns contains GlycINATOR® covalently coupled to agarose beads for deglycosylation of Fc-glycans. IgG is incubated with the GlycINATOR® agarose beads for 30 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® Midispin columns are supplied in 20% EtOH and no preservatives are added.

One Midispin column contains sufficient GlycINATOR® coupled agarose beads to deglycosylate 10 mg IgG.

GlycINATOR® Midispin columns are shipped on ice. GlycINATOR® Midispin columns should be stored at +4-8°C upon arrival.
Do not freeze this product!

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

Additional Materials Required

- Reaction buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Collection tubes: 15 ml conical centrifuge tubes.

Method

- Make sure your antibody is in reaction buffer (See Additional Materials Required above). Prepare a maximum of 10 mg IgG in 0.5-2 ml reaction buffer.
- Lids and bottom caps are used during the incubation. Caps and lids are extra sealed with parafilm during incubation to prevent leakage.
- 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 seal of the spin column and loosen the lid.
 2. Centrifuge the column at 100×g for 1 min to remove storage solution.
 3. Equilibrate the column with 2.5 ml reaction buffer (See Additional Materials Required above).
 4. Centrifuge the column at 100×g for 1 min.
 5. Repeat steps 3 and 4 two times.
 6. Put on the bottom cap (included) to the end of the spin column. Take care to seal it tightly by applying parafilm to prevent leakage.
 7. Immediately add 0.5- 2 ml IgG, maximum 10 mg IgG, in reaction buffer.

8. Re-seal the column with the lid. Take care to seal it tightly by applying parafilm to prevent leakage.
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 30 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 15 ml collection tube (not included).
13. Centrifuge the column at 100 xg for 1 min to elute the sample.

For maximum recovery of the sample:

14. Seal the column with the bottom cap.
15. Add 1 ml reaction buffer.
16. Seal the column with the lid and invert a couple of times.
17. Remove the bottom cap and place the column in a clean 15 ml collection tube (not included).
18. Loosen the top lid and centrifuge the column at 100 xg for 1 min to elute the sample.
19. Repeat steps 14-18 one more time.
20. Pool the eluted fractions.

References

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 α 1-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.

GlycINATOR®

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