Immobilized

GlycINATOR®

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GENOVIS

INSTRUCTIONS FOR PRODUCTS

Immobilized GlycINATOR® Microspin 2 columns
(A0-GL6-010) – Deglycosylation of up to 2 × 0.5 mg IgG

Immobilized GlycINATOR® Microspin 5 columns
(A0-GL6-025) – Deglycosylation of up to 5 × 0.5 mg IgG

Immobilized GlycINATOR® Microspin 10 columns
(A0-GL6-050) – Deglycosylation of up to 10 × 0.5 mg IgG

Immobilized GlycINATOR® Midispin 1 column
(A0-GL6-100) – Deglycosylation of up to 10 mg IgG

Immobilized GlycINATOR® Maxispin 1 column
(A0-GL6-1000) – Deglycosylation of up to 100 mg IgG

Quick Guide (only valid for Microspin columns)

- The Quick Guide (p. 3) is intended for experienced users. First time users of all Immobilized GlycINATOR formats are recommended to follow the detailed protocol (p. 6).
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and slightly open the lid.

Sample Preparation

- Prepare the antibody in 100-300 µl reaction buffer. Max 0.5 mg IgG per column.

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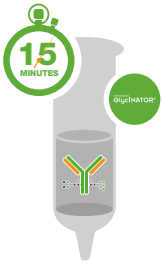
Antibody Deglycosylation – Microspin

1 Equilibration

- Equilibrate the column with 3 x 300 µl reaction buffer. Centrifuge at 200 x g for 1 min.

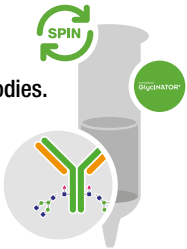
2 Deglycosylation

- Add the antibody to the column and cap the column.
- Incubate at room temperature with end-over-end mixing for 15 min[2].



3 Collection

- Centrifuge at 1000 x g for 1 min to collect the deglycosylated antibodies.
- For maximum recovery, add 100 µl reaction buffer and centrifuge at 1000 x g for 1 min.
- Repeat once.



PRODUCT DESCRIPTION

The Immobilized GlycINATOR (EndoS2) columns contain GlycINATOR covalently coupled to agarose beads for deglycosylation of Fc-glycans without enzyme in the final preparation. IgG is incubated with the GlycINATOR resin for 15-30 min, deglycosylated IgG is then collected by a 1 minute centrifugation step.

GlycINATOR (EndoS2) is an endoglycosidase for deglycosylation of the Fc N-glycan moieties of IgG (1). All IgG Fc 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 (1).

Content and Storage

The Immobilized GlycINATOR columns contain sufficient material to deglycosylate up to 0.5 mg (Microspin), 10 mg (Midispin) or 100 mg (Maxispin) IgG per column. The resin is supplied in 20% EtOH with no preservatives added.

Immobilized GlycINATOR is shipped cold and should be stored at +4-8°C upon arrival.
Do not freeze the product!

Immobilized GlycINATOR is for R&D use only.

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).
- Bottom caps for Midi- and Maxispin columns are included.
- Seal caps and lids of Midi- and Maxispin columns with parafilm during the incubation to prevent leakage.

Additional Materials Required

- Reaction buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Collection tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

Sample Preparation

- Prepare the antibody in the reaction buffer[1] according to Table 1 below.

Table 1. *Preparation of antibodies*

Product Format	Microspin	Midispin	Maxispin
IgG in buffer	100-300 µl	0.5-2 ml	5-10 ml
Max amount IgG/column	0.5 mg	10 mg	100 mg

Deglycosylation of IgG

Protocol parameters for using the different product formats are given in Table 2.

1 Equilibration

- Break off the bottom cap of the column (save the cap for Microspin) and place the column in a collection tube. Loosen the lid.
- Centrifuge for 1 min to remove storage solution.
- Equilibrate the column by adding the reaction buffer and centrifuge the column for 1 min.
- Repeat the equilibration step twice.
- Seal the spin column with the bottom cap.

2 Deglycosylation

- Add the antibody in a volume reaction buffer[1] according to Table 1.
- Seal the column with the top lid.
- Fully suspend the media manually and make sure there is a flow in the column.
- Incubate the column by end-over-end mixing at room temperature for the time indicated in Table 2.

Table 2. *Protocol parameters for the different product formats*

Product Format	Microspin	Midispin	Maxispin
Storage solution removal			
Conical tubes	1.5-2 ml	15 ml	50 ml
Spin	200 x g	100 x g	100 x g
Equilibration			
Add buffer volume	300 µl (x3)	2.5 ml (x3)	10 ml (x3)
Spin	200 x g	100 x g	100 x g
Deglycosylation			
Incubation time[2]	15 min	30 min	30 min
Collection			
Conical tubes	1.5-2 ml	15 ml	50 ml
Spin	1000 x g	100 x g	100 x g
Time	1 min	1 min	2 min
For max recovery			
Add buffer volume	100 µl (x2)	1 ml (x2)	5 ml (x2)
Spin	1000 x g	100 x g	100 x g

3 Collection

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column for the time indicated in Table 2 to recover the deglycosylated antibody.

For Maximum Recovery of the Sample:

- Seal the column with the bottom cap.
- Add reaction buffer according to Table 2.
- Seal the column and invert it a couple of times.
- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column for 1 min to recover the sample.
- Repeat once.
- Pool the collected fractions.

Notes

1. *Immobilized GlycINATOR is compatible with commonly used buffers with pH ranging from 6.0 to 8.0 but the reaction conditions need to be evaluated to ensure efficient deglycosylation.*
2. *The incubation time may be increased if necessary.*

Quality Control

Immobilized GlycINATOR is tested to meet the specifications and lot-to-lot consistency.

Immobilized GlycINATOR is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Product 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 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.

Related Products

GlycINATOR®
Deglycosylation of the IgG Fc domain

IgGZERO®
Deglycosylation of the IgG Fc domain

Legal and Disclaimers

Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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