GENOVIS

GlycINATOR™

Immobilized

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

GlycINATOR™ Immobilized
Microspin 2×0.5mg (A0-GL6-010)
Process 2×0.5mg IgG

GlycINATOR™ Immobilized
Microspin 5×0.5mg (A0-GL6-025)
Process 5×0.5mg IgG

GlycINATOR™ Immobilized
Microspin 10×0.5mg (A0-GL6-050)
Process 10×0.5mg IgG

GlycINATOR™ Immobilized
Midispin 1-10mg (A0-GL6-100)
Process 1-10mg IgG

GlycINATOR™ Immobilized
Maxispin 10-100mg (A0-GL6-1000)
Process 10-100mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A0-GL6

Immobilized Enzyme for Hydrolysis of All Fc N-glycans in Spin Columns

GlycINATOR (EndoS2) is an IgG-specific endoglycosidase that hydrolyzes all glycoforms present at the Fc N-glycosylation sites. The enzyme acts on native IgG and leaves the core GlcNAc intact, with or without fucose. The GlycINATOR Immobilized spin columns contain the GlycINATOR enzyme covalently coupled to agarose beads, for deglycosylation of IgG without contaminating the final preparation with enzyme. GlycINATOR deglycosylates all human IgG subclasses and IgG from many different species, including mouse, rabbit, rat, monkey, sheep, goat, cow, and horse. It removes all Fc glycoforms on IgG, including high-mannose, hybrid, complex, and bisecting type glycans.[1]

GlycINATOR is derived from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 92 kDa.

CONTENT AND STORAGE

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

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

GlycINATOR Immobilized is for R&D use only.

QUALITY CONTROL

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

GlycINATOR Immobilized 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

GlycINATOR™ Lyophilized
Lyophilized enzyme for hydrolysis of all Fc N-glycans

IgGZERO™
Hydrolysis of Fc N-glycans

PNGase F
Hydrolysis of N-glycans

OglyZOR™
Hydrolysis of core 1 O-glycans

1. GlycINATOR has also been reported to hydrolyze glycan moieties from alpha-1-acid glycoprotein.

Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap (save the cap) and loosen the lid (do not remove the lid).
- Bottom caps for Midi- and Maxispin columns are attached by inverting the cap and firmly press it on to the bottom of the column.

Additional Materials Required

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

Hydrolysis of All Fc N-glycans in Spin Columns

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

Sample Preparation
Prepare the antibody in the reaction buffer according to Table 1.

Table 1. Preparation of IgG

	Microspin	Midispin	Maxispin
IgG in buffer[2]	100-300 µl	0.5-2 ml	5-10 ml
Amount IgG	0.5 mg	1-10 mg	10-100 mg

1. Equilibration

1.1 Twist off the bottom cap of the GlycINATOR Immobilized column (save the cap) and place the column in a centrifuge tube. Loosen the lid.

1.2 Centrifuge for 1 min to remove storage solution. Discard the flow-through.

1.3 Place the column in the centrifuge tube.

1.4 Equilibrate the column by adding reaction buffer according to Table 2, and centrifuge for 1 min. Discard the flow-through.

1.5 Perform step 1.4 two additional times.

1.6 Attach the bottom cap.

2. GlycINATOR Immobilized is compatible with commonly used buffers with pH 6.0-8.0, but the reaction conditions need to be evaluated to ensure efficient deglycosylation.

2. Enzymatic Reaction

2.1 Add the antibody in a volume of reaction buffer according to Table 1.

2.2 Seal the column with the lid.

2.3 Fully suspend the media, mix by inversion and make sure there is a flow in the column.

2.4 Incubate the column with end-over-end mixing at room temperature for the time indicated in Table 2.

Table 2. Protocol Parameters for the Different Product Formats

	Microspin	Midispin	Maxispin
Storage Solution Removal			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	100 × g	100 × g
Time	1 min	1 min	1 min
Equilibration			
Add buffer volume	300 µl (×3)	2.5ml (×3)	10 ml (x3)
Spin	200 × g	100 × g	100 × g
Time	1 min	1 min	1 min
Enzymatic Reaction			
Incubation time[3]	15 min	30 min	30 min
Collection of Processed Material			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	1000 × g	100 × g	100 × g
Time	1 min	1 min	2 min
For Maximum Recovery			
Add buffer volume	100 µl (×2)	1 ml (×2)	5 ml (×2)
Spin	1000 × g	100 × g	100 × g
Time	1 min	1 min	1 min

3. Collection of Processed Material

3.1 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.

3.2 Centrifuge according to Table 2 to collect the processed material.

4. For Maximum Recovery of the Sample

4.1 Attach the bottom cap.

4.2 Add reaction buffer according to Table 2.

4.3 Seal the column with the lid and invert it a couple of times.

4.4 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.

4.5 Centrifuge according to Table 2 to collect the processed material.

4.6 Repeat steps 4.1-4.5.

4.7 Pool the collected fractions, including the sample from step 3.2.

3. The incubation time can be increased if necessary.

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