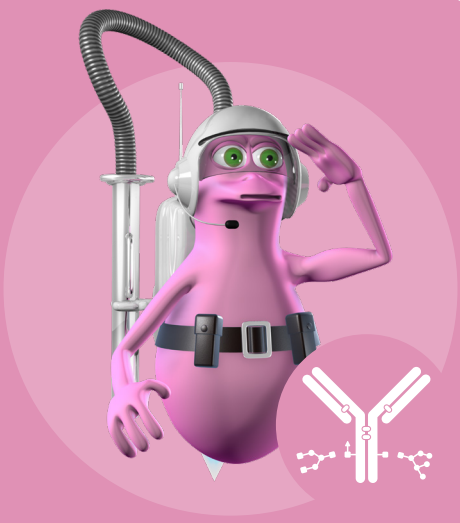
GENOVIS



IgGZERO™

Immobilized

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

IgGZERO™ Immobilized Microspin 2×0.5mg (A0-IZ6-010)
Process 2×0.5mg IgG

IgGZERO™ Immobilized Microspin 5×0.5mg (A0-IZ6-025)
Process 5×0.5mg IgG

IgGZERO™ Immobilized Microspin 10×0.5mg (A0-IZ6-050)
Process 10×0.5mg IgG

IgGZERO™ Immobilized Midispin 1-10mg (A0-IZ6-100)
Process 1-10mg IgG

IgGZERO™ Immobilized Maxispin 10-100mg (A0-IZ6-1000)
Process 10-100mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A0-IZ6

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

IgGZERO (EndoS) is an IgG-specific endoglycosidase hydrolyzing complex N-glycans at the Fc N-glycosylation sites.

The IgGZERO Immobilized spin columns contain the IgGZERO enzyme covalently coupled to agarose beads, for deglycosylation of Fc N-glycans without contaminating the final preparation with enzyme. IgGZERO deglycosylates all human IgG subclasses and IgG from many different species, including mouse, rabbit, rat, monkey, sheep, goat, cow, and horse. The enzyme hydrolyzes glycans from the Fc-domain of IgG, even when other glycosylated proteins are present in the solution. It has limited activity on high-mannose and hybrid-type glycans – for deglycosylation of antibodies carrying these glycoforms, we recommend using GlycINATOR® Immobilized (A0-GL6-010).

IgGZERO is derived from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and has a molecular weight of 112 kDa.

CONTENT AND STORAGE

The IgGZERO 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.

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

IgGZERO Immobilized is for R&D use only.

QUALITY CONTROL

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

IgGZERO 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

PNGase F Immobilized
Immobilized enzyme for hydrolysis of N-glycans in spin columns

IgGZERO™ Lyophilized
Lyophilized enzyme for hydrolysis of complex-type Fc N-glycans

OmniGLYZOR® Kit
A mix of immobilized enzymes in spin columns for hydrolysis of N- and mucin-type O-glycans

Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap (save the cap for Microspin) 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.[1]
- Centrifuge tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

Hydrolysis of Complex-type 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	100-300 µl	0.5-2 ml	5-10 ml
Max amount IgG/column	0.5 mg	10 mg	100 mg

1. Equilibration

1.1 Break off the bottom cap of the IgGZERO Immobilized column (save the cap for Microspin) and place the column in a centrifuge tube. Loosen the lid.

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

1.3 Equilibrate the column by adding reaction buffer and centrifuge for 1 min. Discard the flow-through.

1.4 Perform step 1.3 two additional times.

1.5 Insert the bottom cap.

1. IgGZERO 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 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[2]	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 Insert 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.

2. The incubation time can be increased if necessary.

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