



# 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





## 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.<sup>1</sup>
- 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
<b>Storage Solution Removal</b>			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200×g	100×g	100×g
Time	1 min	1 min	1 min
<b>Equilibration</b>			
Add buffer volume	300 µl (×3)	2.5 ml (×3)	10 ml (×3)
Spin	200×g	100×g	100×g
Time	1 min	1 min	1 min
<b>Enzymatic Reaction</b>			
Incubation time <sup>2</sup>	15 min	30 min	30 min
<b>Collection of Processed Material</b>			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	1000×g	100×g	100×g
Time	1 min	1 min	2 min
<b>For Maximum Recovery</b>			
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.



