

QA0-IZ6-010, QA0-IZ6-025, and QA0-IZ6-050 deGlycIT™ MicroSpin Columns Instructions for Use Deglycosylate up to 0.5mg IgG per column

Product Description

deGlycIT™ MicroSpin Columns are prefilled with IgGZERO® enzyme covalently coupled to agarose. IgGZERO® is an endoglycosidase with a very high specificity for IgG molecules of all species and subclasses.

Contents and Storage

deGlycIT™ MicroSpin column is supplied in 20% EtOH. No preservatives are added. One deGlycIT™ MicroSpin column is sufficient to deglycosylate 0.5mg IgG.

deGlycIT™ MicroSpin is shipped on cold packs and should be stored at 4-8°C upon arrival.

deGlycIT™ Spin Columns are for *in vitro* R&D use only.

Quality Control

IgGZERO® enzyme is tested to ensure lot-to-lot consistency.

IgGZERO® enzyme is tested for sterility on blood agar plates, Sabaraud dextrose agar plates, and in thioglycolate broth.

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Additional Materials Required / Not Provided

- ✓ Deglycosylation buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4
- ✓ Collection tubes: Micro centrifuge tubes (1.5-2ml)

Procedure – IgG Deglycosylation

★ **Make sure your antibody is in deglycosylation buffer.**

1. Break off the bottom plastic cap of the spin column and slightly open the screw cap lid 90° counter-clockwise. **SAVE THE BOTTOM CAP!** Lids and bottom caps are used during the incubation step.
2. Place the spin column in a 1.5-2ml micro centrifuge tube.
3. Centrifuge the spin column at 200 x g for one (1) minute to remove storage solution.
4. Equilibrate the spin column with 300ul of deglycosylation buffer.
5. Centrifuge the spin column at 200 x g for one (1) minute.
6. Repeat steps 4 and 5 two more times.
7. Re-insert the bottom cap of the spin column.
8. Immediately add 100ul of IgG at a maximum concentration of 5mg/ml in deglycosylation buffer.
9. Re-seal the spin column with the lid.
10. Take care to stir and suspend the agarose beads manually and make sure the spin column will flow.
11. Incubate the spin column by end-over-end mixing at room temperature for 15 minutes.
12. Open the spin column lid and remove the bottom cap.
13. Place the spin column in a 1.5-2ml micro centrifuge tube.
14. Centrifuge the spin column at 1,000 x g for one (1) minute to elute the sample.

For maximum recovery of sample:

15. Add an additional 100ul of deglycosylation buffer to the spin column.
16. Place the spin column in a 1.5-2ml micro centrifuge tube.
17. Centrifuge the spin column at 1,000 x g for one (1) minute to elute the sample.
18. Repeat steps 15-17 one more time.

Product Reference

Allhorn M et al. 2008 EndoS from *Streptococcus pyogenes* is hydrolyzed by the cysteine proteinase SpeB and requires glutamic acid 235 and tryptophans for IgG glycan-hydrolyzing activity. *BioMed Central Microbiology* 8: 3.