

# QA0-IZ6-100

## deGlycIT™ MidiSpin Columns Instructions for Use

### Deglycosylate up to 10mg IgG per column

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#### Product Description

deGlycIT™ MidiSpin 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™ MidiSpin column is supplied in 20% EtOH. No preservatives are added. One deGlycIT™ MidiSpin column is sufficient to deglycosylate 10mg IgG.

deGlycIT™ MidiSpin 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: 15ml conical centrifuge tubes

#### 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 15ml conical centrifuge tube.
3. Centrifuge the spin column at 100 x g for one (1) minute to remove storage solution.
4. Equilibrate the spin column with 2.5ml of deglycosylation buffer.
5. Centrifuge the spin column at 100 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. Take care to seal it tightly and apply parafilm around the bottom cap to prevent leakage.
8. Immediately add 0.5-2ml of IgG at a maximum concentration of 20mg/ml in deglycosylation buffer.
9. Re-seal the spin column with the lid. Apply parafilm around the top lid to prevent leakage.
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 30 minutes.
12. Open the spin column lid and remove the bottom cap.
13. Place the spin column in a 15ml conical centrifuge tube.
14. Centrifuge the spin column at 100 x g for one (1) minute to elute the sample.

#### For maximum recovery of sample:

15. Add an additional 1.0ml of deglycosylation buffer to the spin column.
16. Place the spin column in a 15ml conical centrifuge tube.

17. Centrifuge the spin column at 100 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.