deGlycIT™

INSTRUCTIONS

Version 15.1.1

Instructions for product no
A0-IZ6-1000 1 column Deglycosylation of up to 100mg IgG

Product Description

IgGZERO® is an endoglycosidase with a very high specificity for IgG molecules of all species and subclasses.

deGlycIT™ MaxiSpin contains IgGZERO® covalently coupled to agarose beads for deglycosylation of IgG. IgG is incubated with the IgGZERO® agarose beads for 15 min, deglycosylated IgG is then collected by a 1 minute centrifugation step. Since IgGZERO® is immobilized on agarose beads there is no need for extensive purification to remove the enzyme.

Content and storage

Spin column containing IgGZERO® covalently coupled to agarose beads.

deGlycIT™ MaxiSpin are supplied in 20% EtOH and no preservatives are added.

One maxi spin column contains sufficient IgGZERO® coupled agarose beads to deglycosylate 100mg IgG.

deGlycIT™ MaxiSpin is shipped on ice. deGlycIT™ MaxiSpin should be stored at +4-8°C upon arrival.

deGlycIT™ MaxiSpin is for R&D use only.

Quality Control deGlycIT™

deGlycIT™ is tested to ensure lot-to-lot consistency.

deGlycIT™ is tested for absence of microbial contamination with blood agar plates, Sabaraud dextrose agar plates and fluid thioglycolate medium.

Additional Materials Required

- ✓ Cleavage buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4.
- ✓ Collection tubes: 50ml conical centrifuge tubes.

Method

- ✓ Make sure your antibody is in cleavage buffer (See Additional Material Required above).
- ✓ Break off the bottom plastic cap of the spin column.
- ✓ Lids and bottom caps are used during the incubation.
- ✓ Before centrifugation remove the bottom cap and slightly open the lid ~90° counter clockwise.

1. Remove the bottom cap of the spin column and slightly open the screw cap lid ~90° counter clockwise.
2. Put the column in a 50ml conical centrifugation tube.
3. Centrifuge the column at 100×g for 1min to remove storage solution.
4. Equilibrate the column with 10ml cleavage buffer (See Additional Material Required above).
5. Centrifuge the column at 100×g for 1min.

6. Repeat step 4 and 5 two times.
7. Put on the bottom cap on the column. Take care to seal it tightly, apply parafilm around the bottom cap to make sure there is no leakage.
8. Immediately add 5.0-10.0ml IgG at a maximal concentration of 20mg/ml in cleavage buffer.
9. Re-seal the column with the lid. Apply parafilm around the top lid to make sure there is no leakage.
10. Take care to fully suspend the media manually and make sure it is flowing in the column.
11. Incubate the column by end-over-end mixing at room temperature for 30 min. The incubation time can be increased without over digestion of the IgG.
12. Twist open the lid and remove the bottom cap.
13. Place the column in a 50 ml collection tube.
14. Centrifuge the column at 200×g for 1min to elute the sample.

For maximum recovery of your sample:

15. Place the column in a 50 ml collection tube.
16. Add 1.0 ml cleavage buffer.
17. Centrifuge the column at 200×g for 1min to elute the sample.
18. Repeat steps 15-17 one more time. The last centrifugation is done at 400g.

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