FragIT™Z

GENOVIS

INSTRUCTIONS

Version 15.1.1

Instructions for products

FragIT™Z 2 columns	A0-FZ6-010	Digestion of up to 2×0.5mg IgG
FragIT™Z 5 columns	A0-FZ6-025	Digestion of up to 5×0.5mg IgG
FragIT™Z 10 columns	A0-FZ6-050	Digestion of up to 10×0.5mg IgG

Product Description

FragIT™ Z is a resin with FabRICATOR® Z enzyme covalently coupled to agarose beads for subunit fragmentation of mouse IgG2a and IgG3 to generate pure $F(ab')_2$ and Fc fragments without contaminating the final preparation with enzyme. FragIT™ Z digests IgG at one specific site just below the hinge. IgG is incubated with the FragIT™Z resin and fragments are then easily collected by a centrifugation step.

The digestion buffer conditions i.e. NaCl concentration (50-150mM) and pH (6.5-7.5), temperature (20-37°C) and time (1-24h) may need to be optimized for individual antibodies.

FragIT™ Z digests mouse IgG2a and IgG3. For digestion of non-murine IgG, FragIT™ is recommended.

Content and storage

FragIT™ Z Microspin columns contains sufficient material to digest 0.5 mg mouse IgG2a. It is supplied in 20% EtOH with no preservatives added.
FragIT™ Z MicroSpin is shipped on ice and should be stored at +4-8°C upon arrival. Do not freeze the product!

Quality Control

FragIT™ Z is tested to ensure lot-to-lot consistency.
FragIT™ Z is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycolate medium.

Additional Materials Required

- Digestion buffer: 10mM sodium phosphate, 50mM NaCl, pH 6.5*.
- Collection tubes: Micro centrifuge tubes (1.5-2 ml).

** A digestion buffer with 50-150mM NaCl with pH 6.5-7.5 can be used but the digestion time needs to be increased with increasing NaCl concentration and pH.*

Protocol

- Lids and bottom caps are used during the incubation.
- Before centrifugation remove the bottom cap and slightly open the lid ~90° counter clockwise.

Quick Guide

1. Remove lid and bottom cap and centrifuge for 1min at 200xg to remove storage solution.
2. Equilibrate the column with 100µl digestion buffer[1], centrifuge, and repeat 3 times.
3. Add 100µl solution containing the antibody, cap the column and incubate for 1h at room temperature[2].
4. Transfer the column to a micro centrifuge collection tube and remove the lid and bottom cap. Collect the antibody fragments by centrifugation at 1000xg for 1min.

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Detailed Guide - Antibody Fragmentation

1. Prepare the antibody in digestion buffer[1] (See Additional Material Required above).
2. Break off the bottom seal of the column (save the cap) and slightly open the lid ~90° counter clockwise.
3. Place the column in a 1.5-2 ml collection tube.
4. Centrifuge the column at 200×g for 1min to remove storage solution.
5. Equilibrate the column by adding 300µl digestion buffer.
6. Centrifuge the column at 200×g for 1min.
7. Repeat steps 4 and 5 two times.
8. Put on the bottom cap on the column.
9. Immediately add the IgG to be digested in a volume of 100µl at a maximal concentration of 5 mg/ml in digestion buffer[1].
10. Seal the column with the top lid.
11. Take care to fully suspend the media manually and make sure it is flowing in the column.
12. Incubate the column by end-over-end mixing for 1h at room temperature[2].
13. Remove the top lid and the bottom cap.
14. Place the column in a 1.5-2 ml collection tube.
15. Centrifuge the column at 1000×g for 1min to elute the sample.

For maximum recovery of your sample:

16. Place the column in a 1.5-2 ml collection tube.
17. Add 100µl cleavage buffer.
18. Centrifuge the column at 1000×g for 1min to elute the sample.
19. Repeat steps 16-18 one more time.
20. Pool all the eluted fractions.

Notes

1. *A digestion buffer with 50-150mM NaCl with pH 6.5-7.5 can be used but the digestion time needs to be increased with increasing NaCl concentrations and pH.*
2. *Increasing the temperature up to 37°C will increase the digestion efficiency. The incubation time can be increased without over-digestion of the IgG.*