FragIT™ Z kit

GENOVIS

INSTRUCTIONS

Version 15.1.1

Instructions for Products

FragIT™ Z Micro Kit 2 columns	A2-FZ2-005	Digestion and purification of up to 0.5 mg IgG
FragIT™ Z Micro Kit 10 columns	A2-FZ2-025	Digestion and purification of up to 5 x 0.5 mg IgG

Product Description

FragIT™ Z Micro Kit consists of an IgG digestion column, FragIT™ Z, and an affinity purification column, CaptureSelect®

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 fragmentation and purification of non-murine IgG, FragIT™ Kit (A2-FR2-005) is recommended.

The CaptureSelect® columns contains multi species Fc affinity matrix and is an agarose bead with a 13 kDa lama antibody fragment recognizing IgG of multiple species with high affinity. The used ligand is directed towards domains on the Fc part of IgG that enable purification of IgG of, amongst others, human, mouse, bovine, rabbit, rat, goat, horse, and sheep.

Content and storage

FragIT™ Z Micro Kit contains two spin columns

- FragIT™ Z MicroSpin column(s), each column include sufficient material to digest 0.5 mg mouse IgG2a. It is supplied in 20% EtOH with no preservatives added.
- CaptureSelect® MicroSpin column(s) (CaptureSelect® is a trademark of BAC BV Netherlands), each column include sufficient material to purify up to 0.5 mg mouse IgG2a. It is supplied in 20% EtOH with no preservatives added.

FragIT™ Z Micro Kit 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.
- Binding buffer: 10mM sodium phosphate, 150mM NaCl, pH 7.4.
- Collection tubes: Micro centrifuge tubes (1.5-2 ml).
- Elution buffer: 0.1M Glycine pH 3.0 and 1M Tris pH 8.0.

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

Protocol

- The Quick Guide is intended for experienced users. First time user are instructed to follow the detailed protocol.
- Lids and bottom caps are used during the incubation.
- Before centrifugation remove the bottom cap and slightly open the lid ~90° counter clockwise.

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Quick Guide

Fragmentation – FragIT™ Z column

1. Remove lid of the FragIT™ Z column and centrifuge for 1min at 200xg to remove storage solution. Equilibrate the column with 100µl digestion buffer, centrifuge and repeat 3 times.
2. Add the IgG in 100µl digestion buffer[1], at max concentration of 10mg/ml.
3. Cap the column and incubate at room temperature with end-over-end mixing for 1h at room temperature[2].
4. Transfer the column to a micro centrifuge tube and remove the lid and bottom cap. Collect the IgG fragments by centrifugation at 1000xg for 1min.

Purification – CaptureSelect®

5. Remove lid of the CaptureSelect® column and centrifuge for 1min at 200xg to remove storage solution. Equilibrate the column with 100µl binding buffer, centrifuge and repeat 3 times.
6. Add the eluted IgG fragments from step 4
7. Incubate with end-over-end mixing for 30 min at room temperature.
8. Transfer the CaptureSelect® column to a micro centrifuge tube and remove the lid and bottom cap. Collect $F(ab')_2$ antibody fragments by centrifugation at 200xg for 1min.
9. For maximum recovery: Add 100µl binding buffer and centrifuge at 1000xg for 1min. Repeat once.

Elution of Fc fragments – CaptureSelect®

10. Wash the CaptureSelect® column two times with 200 µl binding buffer, centrifuge at 200xg for 1 min.
11. Add 100µl 0.1M Glycine pH 3.0 to the CaptureSelect® column and invert a couple of times.
12. Add 10µl 1M Tris pH 8.0 to a new micro centrifuge collection tube and transfer the CaptureSelect® column to this tube. Elute the Fc fragments by centrifugation at 200xg for 1 min.

Detailed Protocol

Fragmentation

1. Prepare the IgG in digestion buffer (See Additional Material Required above).
2. Break off the bottom seal of the FragIT™ Z 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 digestion buffer.
18. Centrifuge the column at 1000×g for 1min to elute the sample.
19. Repeat steps 16-18 one more time.
20. Pool the eluted fractions.

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Purification

21. Break off the bottom seal of the CaptureSelect® columns (save the cap) and slightly open the lids ~90° counter clockwise.
22. Place the column in 1.5-2 ml collection tubes.
23. Centrifuge the columns at 200×g for 1min to remove storage solution.
24. Equilibrate the columns with 300µl binding buffer.
25. Centrifuge the columns at 200×g for 1min.
26. Repeat step 24 and 25 two times.
27. Seal the spin column with the bottom cap.
28. Immediately add the pooled elution fractions (20) to the CaptureSelect® column.
29. Re-seal the columns with the top lids.
30. Take care to fully suspend the media manually and make sure it is flowing in the columns.
31. Incubate the columns by end-over-end mixing at room temperature for 30min.
32. Remove the lids and the bottom caps.
33. Place the columns in new 1.5-2 ml collection tubes.
34. Centrifuge the columns at 200×g for 1min to elute the sample.

For maximum recovery of your sample:

35. Add 100µl binding buffer to the column.
36. Place the column in new 1.5-2 ml collection tube.
37. Centrifuge the column at 200×g for 1min to elute sample.
38. Repeat step 35-37 one more time. At the final centrifugation centrifuge at 1000×g for 1min.
39. Pool the eluted fractions.

Elution of Fc fragments

40. Re-insert the bottom cap of the CaptureSelect® spin column.
41. Add 100µl 0.1M Glycine pH 3.0.
42. Re-seal the CaptureSelect® spin column with the lid.
43. Take care to fully suspend the media and invert the CaptureSelect® spin column manually a couple of times.
44. Open the lid and remove the bottom cap.
45. Add 10µl 1M Tris pH 8.0 to a new micro centrifuge collection tube (1.5-2 ml) and transfer the CaptureSelect® column to this tube.
46. Centrifuge the CaptureSelect® spin column at 200×g for 1min to elute the sample.
47. Repeat step 40-46 one more time for maximum recovery. At the final centrifugation centrifuge at 1000×g for 1min.

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.*

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