FabALACTICA™ Fab kit

GENOVIS

INSTRUCTIONS

Last revised Nov 2017

Instructions for product no:

A2-AFK-005	2 columns	Digestion and purification of up to 0.5 mg human IgG1
A2-AFK-025	10 columns	Digestion and purification of up to 5 x 0.5 mg human IgG1

Content and storage

FabALACTICA Fab kit Microspin contains two different spin columns:

- Immobilized FabALACTICA Microspin column(s), each column includes sufficient material to digest 0.5 mg hIgG1. It is supplied in 20% EtOH with no preservatives added.
- CaptureSelect™ Fc* Microspin column(s), each column includes sufficient material to purify Fab from 0.5 mg hIgG1. It is supplied in 20% EtOH with no preservatives added.

FabALACTICA Fab kit Microspin is shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the product!**

FabALACTICA Fab kit Microspin is for R&D use only.

Product Description

FabALACTICA Fab kit is used for preparation of pure Fab fragments without contamination by enzyme. The kit involves two steps, digestion of human IgG1 on one column and purification of the Fab fragments using an affinity purification column, Capture Select™ Fc.

The digestion column has a resin with FabALACTICA enzyme covalently coupled to agarose beads for fragmentation of human IgG1 to generate Fab and Fc fragments. Immobilized FabALACTICA digests human IgG1 specifically at ..KSCDKT / HTCPPCP..under physiological reaction conditions thus preserving the immunoreactivity. The digestion is performed at room temperature overnight and there is no risk of overdigestion. Digestion can also be performed at 37 °C to decrease the incubation time, optimization is then required. After incubation with Immobilized FabALACTICA resin the fragments are then easily collected by a centrifugation step.

The Fab fragments are subsequently separated from Fc using the CaptureSelect™ Fc column(s) with multi species Fc affinity resin. The resin consists of a 13 kDa llama antibody fragment recognizing Fc of multiple species with high affinity coupled to agarose beads. After incubation of the digest from the Immobilized FabALACTICA column with Capture Select Fc resin the pure Fab fragments are easily collected by a centrifugation step.

Quality Control

Immobilized FabALACTICA is tested to meet specification. Immobilized FabALACTICA is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Additional Materials Required

- Digestion buffer[1]: 150 mM sodium phosphate, pH 7.0.
- PBS buffer: 10 mM sodium phosphate, 150 mM NaCl, pH 7.4.
- Collection tubes: Micro centrifuge tubes (1.5-2 ml)

Detailed protocol

- Lids and bottom caps are used during the incubation.
- Before centrifugation remove the bottom cap and loosen the lid (do not remove the lid).

* Made with Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Scientific Inc. and its subsidiaries.

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Sample preparation

- Prepare the antibody to be digested in 100 µl[2] digestion buffer / column at a concentration of 5 mg/ml.

Digestion - Immobilized FabALACTICA™ column

Equilibration

1. Break off the bottom seal (save the cap) of the Immobilized FabALACTICA column and loosen the lid.
2. Place the column in a 1.5-2 ml collection tube.
3. Centrifuge the column at 200×g for 1 min to remove the storage solution.
4. Equilibrate the column by adding 300 µl digestion buffer.
5. Centrifuge the column at 200×g for 1min.
6. Repeat steps 4 and 5 two times.
7. Seal the spin column with the bottom cap.

Digestion

8. Immediately add the hIgG1 to be digested in a volume of 100 µl at a concentration of 5 mg/ml in digestion buffer[2].
9. Seal the column with the top lid.
10. Take care to fully suspend the media, mix by inversion and **make sure it is flowing in the column**.
11. Incubate the column by end-over-end mixing overnight (16-18 h) at room temperature. **A good mixing is important for optimal performance.**

Collection of Fragments

12. Remove the bottom cap.
13. Place the column in a 1.5-2 ml collection tube. Loosen the top lid.
14. Centrifuge the column at 1000×g for 1 min to elute the fragments.

For maximum recovery of your sample:

15. Seal the spin column with the bottom cap.
16. Place the column in a 1.5-2 ml collection tube.
17. Add 100 µl PBS buffer.
18. Seal the column and invert the column a couple of times.
19. Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
20. Centrifuge the column at 1000×g for 1 min to elute the sample.
21. Repeat steps 15-20 one more time.
22. Pool all the eluted fractions.

Purification of Fab Fragments – Capture Select™Fc column

Equilibration

1. Break off the bottom seal of the CaptureSelect Fc column (save the cap) and slightly loosen the lid.
2. Place the column in 1.5-2 ml collection tube.
3. Centrifuge the column at 200×g for 1 min to remove storage solution.

4. Equilibrate the column by adding 300 µl PBS buffer.
5. Centrifuge the column at 200×g for 1 min.
6. Repeat step 4 and 5 two times.
7. Seal the spin column with the bottom cap.

Binding of Fc

8. Immediately add the pooled elution fractions from Immobilized FabALACTICA column to the CaptureSelect Fc column.
9. Re-seal the column with the top lid.
10. Take care to fully suspend the media, mix by inversion and make sure it is flowing in the column.
11. Incubate the column by end-over-end mixing at room temperature for 30 min.

Collection of Fab

12. Remove the bottom cap.
13. Place the column in a new 1.5-2 ml collection tube. Loosen the top lid.
14. Centrifuge the column at 200×g for 1 min to elute the Fab fragments.

For maximum recovery of Fab fragments

15. Seal the spin column with the bottom cap.
16. Add 100 µl PBS buffer to the column, seal the column and invert a couple of times.
17. Remove the bottom cap.
18. Place the column in a new 1.5-2 ml collection tube. Loosen the lid.
19. Centrifuge the column at 200×g for 1 min to elute the Fab fragments.
20. Repeat steps 15-19 one more time. Centrifuge at 1000×g for 1 min in the final centrifugation step.
21. Pool the eluted Fab fragments.

Notes

1. Optimal activity is obtained in 100-150 mM sodium phosphate buffers at pH 6.5-7.5. Sodium chloride up to 150 mM can be added without affecting the enzyme activity.
2. The volume should be at least 100 µl / column, but can be increased up to 300 µl / column (Max 0.5 mg hIgG1). The digestion efficiency is likely reduced if concentration is below 0.5 mg/ml.

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