FabALACTICA™ Fab kit

GENOVIS

INSTRUCTIONS

Last revised Dec 2017

Instructions for product no:

A2-AFK-1000	3 columns	Digestion and purification of up to100 mg human IgG1

Content and storage

FabALACTICA Fab kit Maxispin contains three spin columns:

- One Immobilized FabALACTICA Maxispin column which includes sufficient material to digest up to 100 mg hIgG1. It is supplied in 20% EtOH with no preservatives added.
- Two CaptureSelect™ Fc* Maxispin columns, each column which includes sufficient material to purify Fab from 50 mg hIgG1. It is supplied in 20% EtOH with no preservatives added.

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

FabALACTICA Fab kit Maxispin 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 affinity purification columns, 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.
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 columns 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: 50 ml conical collection tubes.
- Parafilm

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).
- Before incubation, seal the bottom cap with Parafilm, or similar, to prevent leakage.

* 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.

Sample preparation

- Prepare the antibody to be digested in digestion buffer, maximum 100 mg hIgG1 in 5-10 ml digestion buffer[2].

Digestion - Immobilized FabALACTICA™ column

Equilibration

1. Break off the bottom seal of the Immobilized FabALACTICA column and loosen the lid.
2. Place the column in a 50 ml collection tube.
3. Centrifuge the column at 100 ×g for 1 min to remove the storage solution.
4. Equilibrate the column by adding 10 ml digestion buffer.
5. Centrifuge the column at 100 ×g for 1 min.
6. Repeat steps 4 and 5 two times.
7. Seal the spin column with the bottom cap. Take care to seal it tightly by applying Parafilm to prevent leakage.

Digestion

8. Immediately add 5-10 ml hIgG1 to be digested, maximum 100 mg hIgG1 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 new 50 ml collection tube. Loosen the top lid.
14. Centrifuge the column at 100 ×g for 2 min to elute the fragments.

For maximum recovery of sample

15. Seal the spin column with the bottom cap.
16. Place the column in a 50 ml collection tube.
17. Add 5 ml PBS buffer.
18. Seal the column with the top lid and invert the column a couple of times.
19. Remove the bottom cap and place the column in a new 50 ml collection tube. Loosen the top lid.
20. Centrifuge the column at 100 ×g for 1 min to elute the sample.
21. Repeat steps 15-20 one more time.
22. Pool the eluted fractions.

Purification of Fab Fragments – Capture Select™Fc columns

Equilibration

1. Break off the bottom seal of the Capture Select Fc columns and slightly loosen the lids.
2. Place the columns in 50 ml collection tubes.

3. Centrifuge the columns at 200 ×g for 1 min to remove the storage solution.
4. Equilibrate the columns by adding 10 ml PBS buffer per column.
5. Centrifuge the columns at 200 ×g for 1 min.
6. Repeat step 4 and 5 two times.
7. Seal the spin columns with the bottom caps. Apply parafilm around the bottom caps to prevent leakage.

Binding of Fc

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

Collection of Fab

12. Remove the bottom caps.
13. Place the columns in new 50 ml collection tubes. Loosen the top lids.
14. Centrifuge the columns at 200 ×g for 1 min to elute the Fab fragments.

For maximum recovery of Fab fragments

15. Seal the spin columns with the bottom caps.
16. Add 2.5 ml PBS buffer to each column, seal the columns and invert a couple of times.
17. Remove the bottom caps.
18. Place the columns in new 50 ml collection tubes. Loosen the lids.
19. Centrifuge the columns at 200 ×g for 1 min to elute the Fab fragments.
20. Repeat steps 15-19 one more time.
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 can be max 10 ml / column (Max 100 mg hIgG1). The digestion efficiency is likely reduced if concentration is < 5 mg/ml.

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