

INSTRUCTIONS

Last revised Nov 2017

Instructions for product no:

A0-AG6-010	2 columns	Digestion of up to 2 × 0.5 mg hlgG1
A0-AG6-050	10 columns	Digestion of up to 10 × 0.5 mg hlgG1

Content and storage

Immobilized FabALACTICA MicroSpin contains:

- Immobilized FabALACTICA MicroSpin columns, each column includes sufficient material to digest 0.5 mg hlgG1. It is supplied in 20% EtOH with no preservatives added.

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

Immobilized FabALACTICA Microspin is for R&D use only.

Product Description

Immobilized FabALACTICA is a resin with FabALACTICA enzyme covalently coupled to agarose beads for fragmentation of human IgG1 to generate Fab and Fc fragments without contaminating the final preparation with enzyme. The human IgG1 is incubated with the Immobilized FabALACTICA resin and the fragments are then easily collected by a centrifugation step.

Immobilized FabALACTICA digests human IgG1 under physiological reaction conditions thus preserving the immunoreactivity. The FabALACTICA enzyme digests human IgG1 at ..KSCDKT / HTCPCP.. 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.

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 buffer¹: 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).

Sample preparation

- Prepare the antibody to be digested in 100 µl² digestion buffer / column to 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 hIlgG1 to be digested in a volume of 100 μ l at a concentration of 5 mg/ml in digestion buffer².
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 \times 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 μ l 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 \times g for 1 min to elute the sample.
21. Repeat steps 15-20 one more time.
22. Pool all the eluted fractions.

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 μ l / column, but can be increased up to 500 μ l / column (Max 0.5 mg hIlgG1). The digestion efficiency is likely reduced if concentration is below 5 mg/ml.

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