Immobilized FabALACTICA™

GENOVIS

INSTRUCTIONS

Last revised Dec 2017

Instructions for product no:

A0-AG6-100 1 column Digestion of 5-10 mg hIgG1

Content and storage

Immobilized FabALACTICA Midispin contains:

- One Immobilized FabALACTICA Midispin column with sufficient material to digest 5-10 mg human IgG1. It is supplied in 20% EtOH with no preservatives added.

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

Immobilized FabALACTICA Midispin 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 / HTCPPCP.. The digestion is performed at room temperature overnight and there is no risk of overdigestion.

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: 15 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.

Sample preparation

- Prepare the antibody to be digested in digestion buffer, maximum 10 mg hIgG1 in 1-2 ml digestion buffer[2].

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 15 ml collection tube.
3. Centrifuge the column at 100 ×g for 1 min to remove the storage solution.

4. Equilibrate the column by adding 2.5 ml digestion buffer.
5. Centrifuge the column at 100 ×g for 1min.
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 1-2 ml hIgG1 to be digested, maximum 10 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 15 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 15 ml collection tube.
17. Add 1 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 15 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 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 can be added up to 150 mM without affecting the enzyme activity.
2. The volume should be at least 1 ml / column, but can be increased up to 2 ml / column (Max 10 mg hIgG1). The digestion efficiency is likely reduced if concentration is < 5 mg/ml.

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