
INSTRUCTIONS

Last revised Dec 2017

Instructions for product no:

A0-AG6-1000 1 column Digestion of up to 100 mg hlgG1

Content and storage

Immobilized FabALACTICA Maxispin contains:

- One Immobilized FabALACTICA Maxispin column with sufficient material to digest up to 100 mg human IgG1. It is supplied in 20% EtOH with no preservatives added.

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

Immobilized FabALACTICA Maxispin 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 / HTPPCP.. 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 buffer¹: 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.

Sample preparation

- Prepare the antibody to be digested in digestion buffer, maximum 100 mg hlgG1 in 5-10 ml digestion buffer².

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 50 ml collection tube.
3. Centrifuge the column at 100 xg 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 hlgG1 to be digested, maximum 100 mg hlgG1 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 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 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.

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 can be max 10 ml / column (Max 100 mg hlgG1). The digestion efficiency is likely reduced if concentration is < 5 mg/ml.

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