GENOVIS

AlligBAITOR™

Affinity Purification

STORE AT
+4-8°C

FOR RESEARCH USE ONLY

Instructions for Use

AlligBAITOR™ Affinity Purification
Microspin 4 × 0.2 mg (A4-AB6-008)
Binds 4 × 0.2 mg Ig

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-A4-AB6

Affinity Resin for All Ig Capture and Antigen Release

AlligBAITOR is an immunoglobulin-binding protein that binds all human immunoglobulin isotypes and subclasses. AlligBAITOR Affinity Purification enables immunoglobulin depletion from human serum and simultaneous, downstream, proteomic characterization of captured immunoglobulins.

AlligBAITOR is active in a wide range of physiological relevant buffer conditions, including PBS and TBS, in a salt concentration up to 500 mM and in the pH range 5.5–8.5.

BINDING CAPACITY

One AlligBAITOR Affinity Purification column binds ≥ 90% of Ig from 10 µl human serum when incubated in PBS buffer at RT for 30 minutes.

CONTENT AND STORAGE

AlligBAITOR Affinity Purification contains four AlligBAITOR microspin columns. The columns are supplied in 20% ethanol, with no preservatives added. One column contains sufficient material to bind 0.2 mg human immunoglobulin. The columns should be stored at +4-8°C upon arrival.
Do not freeze the columns!

AlligBAITOR Affinity Purification is for R&D use only.

QUALITY CONTROL

AlligBAITOR Affinity Purification is tested to meet the specifications and lot-to-lot consistency.

AlligBAITOR Affinity Purification is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

YOU MIGHT ALSO BE INTERESTED IN

FabRICATOR®
Below hinge digestion of IgG (IdeS)

IgMBRAZOR™
Digestion of IgM

IgASAP™
Digestion of IgA

Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).

Additional Materials Required

- Binding buffer: PBS (10–150 mM sodium phosphate, 150 mM NaCl, pH 7.4) or TBS (10–50 mM Tris, 150 mM NaCl, pH 7.0–8.0).
- Wash buffer: 50 mM Tris, 1.5 M NaCl, 1.5 M urea, pH 8.0.
- Elution buffer: 8 M Guanidine hydrochloride pH 8.5 buffered aqueous solution.
- Microcentrifuge tubes: 1.5–2 ml.

Enrichment, Depletion, and Fractionation of Immunoglobulins

Sample Preparation
Prepare the protein sample solution in 150 µl[1] binding buffer/column. One column will efficiently bind all immunoglobulins (Ig) from 10 µl serum[2].

1. Equilibration

1.1 Break off the bottom cap of the AlligBAITOR Microspin column[3] (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.

1.2 Centrifuge at 200 × g for 1 min to remove the storage solution. Discard the flow-through.

1.3 Equilibrate the column by adding 300 µl binding buffer and centrifuge at 200 × g for 1 min. Discard the flow-through.

1.4 Perform step 1.3 two additional times.

1.5 Insert the bottom cap.

2. Binding of Ig and Elution of Non-Ig Fraction

2.1 Add the sample solution to the column.

2.2 Seal the column with the lid.

2.3 Fully suspend the media, mix it by inversion and make sure there is a flow in the column.

2.4 Incubate the column with end-over-end mixing at room temperature for 30 min. A good mixing is important for optimal performance.

2.5 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

2.6 Centrifuge at 1000 × g for 1 min to collect the flow-through as the Ig-depleted sample.

2.7 Insert the bottom cap.

1. The volume should be at least 100 µl/column and can be increased up to 500 µl/column
2. For purification of a single immunoglobulin isotype, one microspin column provides a binding capacity of up to 150 µg IgM, 225 µg IgA, or 225 µg IgG. Although IgD and IgE quantities have not been evaluated, capacities of at least 200 µg per column are anticipated based on their structural characteristics.
3. Four AlligBAITOR Microspin columns are included. Each column contains sufficient material to bind 0.2 mg human immunoglobulin.

3. Washing

3.1 Wash the column by adding 300 µl binding buffer. Seal the column with the lid. Mix by inversion.

3.2 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

3.3 Centrifuge at 200 × g for 1 min.

3.4 Insert the bottom cap.

3.5 Perform steps 3.1-3.4 two additional times using wash buffer[4].

3.6 Perform a final wash step 3.1-3.4 using binding buffer.

4. Elution of Ig-fraction

4.1 Add 100 µl elution buffer to the column.

4.2 Seal the column with the lid and mix by tapping the tube and incubate for 4 min at RT.

4.3 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.

4.4 Centrifuge at 1000 × g for 1 min to collect the eluted Ig material.

Optional: Insert the bottom cap and repeat steps 4.1–4.4 to improve recovery.

4. Ig binding to the resin is highly robust, allowing wash conditions to be optimized for the sample matrix, including the use of low-pH buffers, 2 M guanidine hydrochloride (Gd-HCl), and other stringent wash conditions.

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