ChroSpin - ProteinA/G

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ABSTRACT

Purification Guide for the Isolation of Antibodies with ChroSpin Columns by DALEX Biotech.

Easy and quick small scale antibody purification from various sources and species.

Each ChroSpin column has a binding capacity of > 2 mg (tested with human polyclonal Ig, binding varies between species and clones).

The proprietary resin does not shrink or swell in aqueous buffers.

High pressure stability.

pH stability short term 2 - 8, long term 3 - 8.

Excellent thermal stability up to 15 minutes at 80 °C in aqueous buffers at neutral pH.

Can be dried for long term storage (80 °C for > 2 h).

EXTERNAL LINK

https://dalex-biotech.com/

PROTOCOL STATUS

Working
Official product protocol by DALEX Biotech.

GUIDELINES

For optimal binding and purity, the pH of the sample should be 7.5-8.5 and should contain 150-300 mM NaCl. An easy way to achieve this is by adding 1/11 volume 0.5 M Tris, 2 M NaCl (pH 8.0) to your sample.

Purification works best with an antibody concentration of 2-3 mg/ml in your sample.

It is advisable that all fractions are collected (Sample, flow through, wash, and eluate) in separate tubes for analysis, e.g. SDS-PAGE.

MATERIALS TEXT

Materials provided in the kit:
ChroSpin columns
Wash buffer
Elution buffer
Neutralization buffer
Sanitization solution

Materials not provided in the kit:
Tween-20
0.5 M Tris, 2 M NaCl (pH 8.0)
Deionized water
20 % ethanol

SAFETY WARNINGS

The buffers in the kit include sodium azide (CAS No. 26628-22-8) as a preservative.
For safety information on this chemical(s) check http://www.dguv.de/ifa/gestis-database

BEFORE STARTING

Make sure your sample is free of particulate matter. You can remove particles by centrifugation or filtration (0.45 µm).

What do you want to do?

1 Do you want to purify antibodies or sanitize your column?
Please choose below.

step case

Purification

no description provided

Equilibration

2 Remove the bottom cap, add 500 µl wash buffer to the column, close the lid, invert sharply 3 - 5 times, and centrifuge 30 - 60 seconds at 1000 g .

NOTE
If you work with a used column, remove the storage solution by centrifugation first.

Load and Wash

3 Add up to 500 µl sample to the top of the column and incubate the column with end-over-end mixing or occasional inversion for 3 minutes.
00:03:00

NOTE
For fast binding antibodies the incubation time can be decreased to 30 seconds. For slow binding antibodies the incubation time can be increased to 10 minutes. Longer incubation time is also possible, however, this will also increase background.

For optimal binding and purity, the pH of the sample should be 7.5 - 8.5 and should contain 150 - 300 mM NaCl. An easy way to achieve this is by adding 1/11 volume 0.5 M Tris, 2 M NaCl (pH 8.0) to your sample.

Purification works best with an antibody concentration of 2 - 3 mg in your sample.

4 Centrifuge the column for 30 - 60 seconds at 1000 g and place the column into a clean receiver tube.

NOTE
It is advisable to keep the flow-through and wash fractions for later analysis, e.g. SDS-PAGE.

5 Add 500 µl wash buffer to the column, close the lid, and invert 5 times. Centrifuge the column for 30 - 60 seconds at 1000 g and empty the receiver tube.

6 Repeat the previous step.
go to step #5

NOTE

For increased purity, repeat the washing step up to 5 times.

In case of unspecific hydrophobic and/or ionic interactions include up to 1 % Tween-20 and/or up to 0.5 M NaCl in the wash buffer.

Elution

7 Place the column into a clean receiver tube, add 200 µl elution buffer to the column, close the lid, and invert three times. Centrifuge the column for 30 - 60 seconds at 1000 g.

8 Leave receiver tube in place. Add 200 µl elution buffer to the column, close the lid and centrifuge for 30 - 60 seconds at 1000 g.
Repeat once more.

9 Add two drops of neutralization solution to the eluate and mix gently.

Cleaning and Storage

10 Wash the column by adding 500 µl elution buffer and centrifuge at 1000 g for 30 - 60 seconds. Repeat this step with 500 µl wash buffer and 500 µl deionized water. Then, close the bottom of the column, add 500 µl 20 % ethanol or wash buffer (contains 0.05 % (w/v) sodium azide). Close the lid and store at room temperature or at 4 - 8 °C.

Alternative for long-term storage:
Dry the open (top and bottom) column in an oven at 80 °C for at least 2 hours or over night. Make sure the bottom stopper is completely dry, too. Put on the column's outlet, close the lid and store the column closed at room temperature.

Sanitization

step case

Sanitization

After five purification cycles or after a detectable decrease in capacity a sanitization of the column is recommended.

2 After purification: wash the column with 500 µl elution buffer and centrifuge the column for 30 - 60 seconds at 1000 g. Then, repeat with 500 µl wash buffer.
If the column was stored before: remove the bottom cap and centrifuge the column for 30 - 60 seconds at 1000 g. Then, add 500 µl of wash buffer and centrifuge the column for 30 - 60 seconds at 1000 g.

3 Close the bottom of the column and add 500 µl sanitization solution. Close the lid and incubate for one hour at RT.