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ChroDrip - ProteinG

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Abstract

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

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

Each ChroDrip column has a binding capacity of > 15 mg/ml (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).

Guidelines

For optimal binding and purity, the pH of the sample should be 7-8 and should contain 150-300 mM NaCl. An easy way to achieve this is by adding 1/11 volume 10x PBS to your sample.

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

Materials

Materials provided in the kit:
ChroDrip column
Wash buffer
Elution buffer
Neutralization buffer
Sanitization buffer

Materials not provided in the kit:
Tween-20
10x PBS
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 start

Removal of particulate matter from the sample by centrifugation or filtration (0.45 µm) is recommended.

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

STEP CASE

Purification 7 steps

Equilibration

2 Remove the bottom and top cap and add 5 column volumes (bed volume is written on the column) of wash buffer to the column.

Note

If you work with a used column, drain the storage solution first.

Load and Wash

3 Add your sample to the top of the column and let it flow through by gravity.

Note

For optimal binding and purity, the pH of the sample should be 7 - 8 and should contain 150 - 300 mM NaCl. An easy way to achieve this is by adding 1/11 volume 10x PBS to your sample.

Removal of particulate matter from the sample by centrifugation or filtration (0.45 µm) is recommended.

For slow or weak binding antibodies collect the flow through and apply it again.

Load and Wash

4 Add 5 column volumes of wash buffer and wait until it has drained. Repeat once more.

Note

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

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

5 Add 0.75 column volumes of elution buffer to the column.

Note

This fraction does not contain the target protein. The small amount of elution buffer replaces most of the wash buffer in the column. This "pre-elution step" will result in a more concentrated eluate.

6 Place a clean tube under the column. Add 3 times 3 column volumes of elution buffer to the column, collect each fraction in a separate tube. Wait inbetween the elution steps until the buffer has drained completely.

Note

For more concentrated eluates, elute 8 times with one column volume and collect each fraction in a separate tube. Determine in which fraction(s) most of the protein is contained and combine these.

7 Add neutralization solution to the eluate. For each milliliter of eluate add three drops neutralization solution.

Cleaning and Storage

8 Wash the column successively with 5 column volumes elution buffer, 5 column volumes wash buffer and 5 column volumes deionized water. Then, add 10 column volumes 20 % ethanol or wash buffer (contains 0.05 % (w/v) sodium azide). Wait until half of the buffer

has drained. Close the top lid and then the bottom stopper. 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.