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ChroPack - ProteinA/G

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1 Works for me dx.doi.org/10.17504/protocols.io.72phqdn

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ABSTRACT

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

With ChroPack Protein A/G columns you can quickly isolate monoclonal and polyclonal antibodies from various sample materials. Antibodies bind with high specificity to the recombinant protein G whose BSA binding site has been removed. The ChroPack columns can be operated with peristaltic pumps and common FPLC systems. The Luer-Lock connections make changing the column particularly easy.

Each ml bed volume has a binding capacity of > 15 mg (tested with polyclonal human Ig, the binding capacity varies according to species and clones).

Up to 98 % purity in a single purification step.

No swelling/shrinking of the matrix in aqueous buffers.

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

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

Suitable for all common low-pressure systems.

GUIDELINES

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

- FPLC-System with Luer-Lock connections
- ChroPack column
- Wash buffer
- Elution buffer
- Sanitization solution (optional)

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

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

What do you want to do?

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

step case

Purification

Equilibration

2 Connect the column to your FPLC system. Set the flow rate to 1 bed volume per minute. Wash the column with 5 volumes deionized water (bed volume is written on the column).

A dry column can be directly connected to the FPLC system without special precautions. The air will be forced out through the bottom outlet.

3 Equilibrate the column with 5 to 10 bed volumes wash buffer.

Load and Wash

4 Load the sample onto the column.

For optimal binding and purity, the pH of the sample should be between 6 - 8 and should contain 150 - 300 mM NaCl. Ideally, the optimal pH value for binding must be determined.
A good start is to add 1/11 volume 10x PBS or 0.5 M Tris, 2 M NaCl (pH 8.0) to your sample.
If the yield is lower as expected try to decrease the pH by adding sodium acetate buffer or increase the pH by adding phosphate buffer.

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.

5 Wash the column with 10 to 20 bed volumes.

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

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

6 Elute with 10 bed volumes elution buffer and collect fractions of 0.5 to 1 bed volumes.

7 Add neutralization solution (1 M TRIS HCl pH 8.5) to the eluate. Add 100 µl neutralization solution to 900 µl eluate.

Cleaning and Storage

8 Wash the column successively with 5 column volumes of elution buffer, 5 column volumes wash buffer and 5 column volumes water. Then, wash with 5 column volumes 20 % ethanol or wash buffer containing 0.05 % (w/v) sodium azide. Close the top lid and then the bottom stopper. Store at room temperature or at 4 - 8 °C.

Sanitization

step case

Sanitization

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

2 Connect the column to your FPLC system and flush with 5 bed columes water, elution buffer and wash buffer (without urea). All steps are at a flow rate of 1 bed volume per minute.

3 Flush with 5 bed volumes sanitization solution, stop the pump and wait 1 hour.