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ChroPlate - ProteinA V.2

Alexandra Ehl[1], David Frommholz[1], Nadine Stefanczyk[1]

[1]DALEX Biotech

1 Works for me dx.doi.org/10.17504/protocols.io.8qphvvn

David Frommholz
DALEX Biotech

ABSTRACT

Purification Guide for the Isolation of Antibodies with ChroPlate Filtration Plates by DALEX Biotech.

Easy and quick high throughput antibody purification from various sources and species.

Each well of the ChroPlate has a binding capacity of > 1 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/

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. For screening of binding condition for e.g. a monoclonal antibody this parameters might be varied.

Purification works best with an antibody concentration of 1-2 mg/ml in your sample.

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

MATERIALS TEXT

Materials provided in the kit:
ChroPlate
Dummy plate
Wash buffer
Elution buffer
Neutralization buffer

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

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).

How do you want to purify?

1 It is advisable to purify by centrifugation and not by vacuum filtration because of excessive frothing.

step case

Centrifugation

Equilibration

2 Add 500 µl wash buffer to each well, place the ChroPlate on top of a deep-well plate, and centrifuge 2 minutes at 400 g in a swing-out rotor. For counterbalance of the centrifuge a dummy filter plate is included in the kit.

Load and Wash

3 Place the ChroPlate on a clean deep-well plate. Add up to 1.5 ml sample to every well. Centrifuge 5 minutes at 400 g in a swing-out rotor.

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

The centrifugation time depends on the sample's volume and viscosity. Volumes larger than 1 ml and viscous samples e.g. serum usually require more than 5 minutes centrifugation time.

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.

4 Empty the deep-well plate or place the ChroPlate on a clean one. Add 500 µl wash buffer to each well and centrifuge 2 minutes at 400 g in a swing-out rotor.

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.

5 Repeat the previous step.

For increased purity, repeat the washing step a third time.

Elution

6 Place the ChroPlate on a clean deep-well plate. Add 300 µl elution buffer to each well and centrifuge 2 minutes at 400 g in a swing-out rotor.
Repeat two more times.

7 Add one drop of neutralization solution to every well of the deep-well plate and mix gently.