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ChroDrip - IMAC

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

[1]DALEX Biotech

Anonymous
DALEX Biotech

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Abstract

Purification Guide for the Isolation of Histidine-tagged Proteins with ChroDrip Columns by DALEX Biotech.

ChroSpin-IMAC by DALEX Biotech offers a robust and convenient way to isolate polyhistidine-tagged protein from bacterial, mamalian, and insect cell cultures.
Immobilized metal affinity chromatography (IMAC) is based on the interaction of the imidazole ring of histidine with transition metal ions immobilized on a solid support. Recombinant proteins with a 3 - 10 histidine fusion tag bind to these metal ions while unwanted proteins are removed by washing with excess binding buffer. Elution of the target protein is achieved by the addition of imidazole, EDTA or a low pH.

Easy and quick small scale fusion protein purification from various sources.

Low metal leaching from column.

Tolerates reducing reagents (e.g. DTT up to 10 mM).

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 120 °C in aqueous buffers at neutral pH.

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

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

Materials provided in the kit:
ChroDrip column
Wash buffer
Elution buffer
Nickel solution
Cobalt solution
Sanitization solution

Materials not provided in the kit:
DNase/lysozyme
Denaturing wash buffer
Denaturing elution buffer
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

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

1 How do you want to purify your protein? Do you want to prepare your column for reuse or sanitize it?
Please choose below.

STEP CASE

Native Purification 10 steps

If you try to purify your protein for the first time or you already know that your protein is in the soluble fraction and the his-tag is accessible, use the "Native Purification" protocol (buffers are included in the kit).

Sample Preparation

2 Determine the weight of the frozen bacterial pellet and thaw at room temperature.
Resuspend the pellet in wash buffer by pipetting. For every gramm of pellet add 3 - 5 milliliters buffer.
Add lysozyme and DNaseI (not included in the kit)
[M] 0.01 Mass / % volume 0.1mg/ml each
Alternatively, use ultrasonication according to the instructions of your instrument manufacturer and skip the next step.

Note

Pre-chill an appropriate centrifuge to 4 °C.

3 Incubate for 15 minutes with gentle end-over-end mixing, stirring, or rocking at room temperature.
If your target protein is known to undergo proteolytic degradation or rapid denaturation, incubate at 4 - 8 °C for 30 minutes.
22 °C 00:15:00
or
4 °C 00:30:00

4 Centrifuge the lysate for 30 minutes at >10.000 g at 4 °C .

Carefully transfer the supernatant to a fresh tube.

Note

During centrifugation you can already proceed with the equilibration of the column.

Equilibration

5 If you start with a dry column, add 5 column volumes deionized water (bed volume is written on the column) and wait until it has drained. Add 0.5 column volumes of nickel or cobalt solution, let the solution drain and add another 5 column volumes of deionized water.

When starting with a column which has been stored in storage solution, drain that solution first.

Note

Apart from Ni^{2+} and Co^{2+}, you can also use Cu^{2+} or Zn^{2+}.

The affinity of histidine towards the metal ions is in the order Cu > Ni > Zn > Co. However, the specificity is in the invers order, i.e. copper will most likely result in best yields but with lower purity. In comparison, cobalt will result in a better purity but also lower yields.

6 Add 5 column volumes of wash buffer and wait until it has drained.

Load and Wash

7 Add the cleared lysate to the column and wait until it has drained.

Note

Collect the flow through and wash fractions in separate tubes for later analysis, e.g. SDS-PAGE

8 Add 5 column volumes of wash buffer, wait until it has drained and repeat once more.

Note

For an increase in purity repeat this step a third time.

Elution

9 Add 0.75 column volumes of elution buffer to the column and let it drain.

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.

10 Add 3 times 3 column volumes of elution buffer to the column. 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 into a separate tube. Determine which fractions contain most of the protein and combine these.

Cleaning and Storage

11 Wash the column successively with 5 column volumes of elution buffer, 5 column volumes wash buffer and 5 column volumes 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. Close the column's outlet

and the lid. Store at room temperature.