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ChroPack - IMAC

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Abstract

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

ChroPack-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 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.

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

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

Before start

Make sure your sample is free of particulate matter. You can remove particles by centrifugation and/or filtration (0.45 µm). Degassing of all buffers is advisable.

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 Purifcation 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.

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 to a concentration of 0.1 mg/ml each.

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.
00:15:00 at RT or 00:30:00 at 4 °C

4 10.000 x g , or higher 4 °C

5 Filter the supernatant with a 0.45 µm filter.

Equilibration

6 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).

Pump 0.5 column volumes of nickel or cobalt solution followed by another 5 bed volumes of deionized water.

Note

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

Note

Apart from Ni2+ and Co2+, you can also use Cu2+ or Zn2+.

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.

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

Load and Wash

8 Load the cleared lysate onto the column.

9 Wash the column for 10 to 20 bed volumes.

Elution

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

11 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