Oct 25, 2019

ChroPack - IMAC

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

[1]DALEX Biotech

1 Works for me dx.doi.org/10.17504/protocols.io.72hhqb6

David Frommholz
DALEX Biotech

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 TEXT

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

BEFORE STARTING

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.

What do you want to do?

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

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.

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.

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

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

Sample Preparation

step case

Denaturing Purification

If native purification failed or you already now that your protein is expressed in inclusion bodies or the his-tag is sterically inaccessible, use the "Denaturing Purification" protocol.

2 Prepare Buffers:

Wash Buffer:

[M]**0.05 Molarity (M) Sodium dihydrogen phosphate**

[M]**8 Molarity (M) Urea**

[M]**0.3 Molarity (M) Sodium chloride**

adjust to pH 8.0 with NaOH

Elution Buffer:

[M]**0.05 Molarity (M) Sodium dihydrogen phosphate**

[M]**8 Molarity (M) Urea**

[M]**.3 Molarity (M) Sodium chloride**

[M]**0.25 Molarity (M) Imidazole**

adjust to pH 8.0 with NaOH

3 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.

Pre-chill an appropriate centrifuge to 4 °C.

4 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**

5 **10.000 x g or higher** **4 °C**

Solutions

step case

Column Regeneration

ChroDrip-IMAC columns can be reused up to ten times without major loss in binding capacity. To completely clean and recharge your column use this protocol.

2 Solutions needed:

- Deionized water
- Cleaning solution 1: 6 M Guanidine chloride, 0.2 M Acetic acid
- Cleaning solution 2: 1 % Sodium dodecyl sulfate (SDS)
- Cleaning solution 3: > 95 % Ethanol
- Cleaning solution 4: 0.1 M Ethylenediaminetetraacetic acid (EDTA)
- Cleaning solution 5: 0.2 M Acetic Acid
- Metal solution: 0.1 M Nickel(II) sulfate (or other metal ion of your choice)
- 20 % (v/v) Ethanol

Wash and Clean

3 Connect the column to your FPLC system and flush the column with 5 bed volumes water.

4 Pump 5 column volumes of cleaning solution 1 to 5, one after another, with 10 bed volume washes with deionized water in between each of the cleaning solutions.

Recharge and Storage

5 Wash the column one more time with 5 column volumes of deionized water. Then, pump 1 column volume of the metal solution. Wash with 10 column volumes of water

Sanitization

step case

Column 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.

01:00:00

4 Flush with 10 bed volumes water.

Recharge and Storage

5 Pump 0.5 bed volumes metal solution.