ChroDrip - IMAC

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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).

EXTERNAL LINK

https://dalex-biotech.com

PROTOCOL STATUS

Working
Official product protocol by DALEX Biotech.

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

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 STARTING

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

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 Purification

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) 0.01 Mass/Volume Percent 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.

Buffer 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 (buffers are NOT included in the kit).

2 **Wash Buffer**

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

4 Molarity (M) **Urea**

0.3 Molarity (M) **Sodium chloride**

Adjust to pH 8.0 with NaOH

Elution Buffer

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

4 Molarity (M) **Urea**

0.3 Molarity (M) **Sodium chloride**

0.250 Molarity (M) **Imidazole**

Adjust to pH 8.0 with NaOH

NOTE
Buffers containing urea are not stable at room temperature. Prepare freshly, use the buffers on the same day or store in appropriately sized aliquots at -20 °C for no longer than two months. Do not freeze and thaw the buffer more than once.

Sample Preparation

3 Determine the weight of the frozen bacterial pellet and thaw on ice.
Resuspend the pellet in binding buffer by pipetting. For every gramm of pellet add 5 milliliters buffer.
Add lysozyme and DNaseI (not included in the kit) 0.01 Mass/Volume Percent **0.1mg/ml each**
Alternatively use ultrasonication according to the instuctions of your instrument manufacturer.

NOTE
Pre-chill an appropriate centrifuge to 4 °C.

4 Incubate for 30 minutes with gentle end-over-end mixing, stirring or rocking at room temperature.
If your target protein is known to undergo proteolytic degradation incubate at 4 - 8 °C for 60 minutes.
22 °C 00:30:00
or
4 °C 01:00:00

5 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.

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
- 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 Remove the bottom cap and drain any storage solution.

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

Final Wash and Recharge

5 Wash the column one more time with 5 column volumes of deionized water. Then, add 1 column volume of the metal solution, let it drain and wait for 5 minutes.
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 After a purification: wash the column with 5 column volumes of elution buffer and 5 column volumes of wash buffer.

If the column was stored in storage solution: let the buffer drain and wash with 5 column volumes of wash buffer.

3 Add 5 column volumes of sanitization solution, let 1 column volume drain and then close the column with the top and bottom cap. Incubate for one hour at RT.

4 Drain the sanitization solution and wash the column with 10 column volumes of deionized water.

Storage

5 Add approximately 5 column volumes of 20 % ethanol or wash buffer (contains 0.05 % (w/v) sodium azide), let 1 column volume drain, close

the top and bottom cap and store 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. Put on the column's outlet, close the lid and store the column closed at room temperature.