ChroSpin - IMAC

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dx.doi.org/10.17504/protocols.io.tv3en8n

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ABSTRACT

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

ChroSpin-IMAC by DALEX Biotech offers a robust and convenient way to isolate polyhistidine-tagged proteins from bacterial, mammalian, 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:
ChroSpin columns
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 now 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 it at room temperature.
Resuspend the pellet in wash buffer by pipetting. For every gram 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 instuctions 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 spin column.

Equilibration

5 Add 500 µl of nickel or cobalt solution, close the lid, and incubate 1 minute with end-over-end mixing.
00:01:00

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 Remove the bottom lid.
Centrifuge the column at 1000 g for 30 - 60 seconds.
Discard the flow through.

7 Add 500 µl deionized water, close the lid, invert sharply 3 - 5 times, and centrifuge the column at 1000 g for 30 - 60 seconds. Discard the flow through.
Repeat once more.

8 Add 500 µl wash buffer, close the lid, invert sharply 3 - 5 times, and centrifuge the column at 1000 g for 30 - 60 seconds.

Load and Wash

9 Place the column into a fresh collection tube. Add up to 500 µl cleared lysate, close the lid, and incubate 5 minutes with end-over-end mixing. Centrifuge the column at 1000 g for 30 - 60 seconds.

NOTE
Repeat the loading step when your sample volume is larger than 500 µl. This is particularly recommended when your target protein has low expression levels.

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

10 Place the column into a fresh collection tube. Add 500 µl wash buffer, close the lid, and invert sharply 3 - 5 times. Centrifuge the column at 1000 g for 30 - 60 seconds and empty the collection tube.

Repeat once more without changing the collection tube.

NOTE
For increased purity, repeat the washing step up to 5 times.

Elution

11 Place the column into a fresh collection tube. Add 200 µl elution buffer to the column. Centrifuge the column at 1000 g for 30 - 60 seconds. Without changing the collection tube, repeat this elution step two more times.

Cleaning and storage

12 Wash the column by adding 500 µl elution buffer and centrifuge at 1000 g for 30 - 60 seconds. Repeat this step with 500 µl wash buffer and 500 µl deionized water. Then, close the bottom of the column, add 500 µl 20 % ethanol or wash buffer (contains 0.05 % (w/v) sodium azide). Close the lid and store at room temperature or at 4 - 8 °C.

Buffer Preparation

step case

Denaturing Purification

If native purification failed or you already know 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 it on ice.
Resuspend the pellet in wash buffer by pipetting. For every gram 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.

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 spin column.

Solutions

step case

Column Regeneration

ChroSpin-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.

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- 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 by centrifugation at 1000 g for 1 minute.

4 Use 500 µl of each cleaning solution, one after another, with two 500 µl washes with deionized water in between each of the cleaning solutions, i.e.:

1. Add 500 µl of cleaning solution 1, invert sharply 4-5 times, centrifuge 1 minute at 1000 g, discard flow through.
2. Add 500 µl of deionized water, invert sharply 4-5 times, centrifuge 1 minute at 1000 g, discard flow through.
3. Add 500 µl of deionized water, invert sharply 4-5 times, centrifuge 1 minute at 1000 g, discard flow through.
4. Add 500 µl of cleaning solution 2, invert sharply 4-5 times, centrifuge 1 minute at 1000 g, discard flow through.
5. Add 500 µl of deionized water, invert sharply 4-5 times, centrifuge 1 minute at 1000 g, discard flow through.
6. ...

Final Wash and Recharge

5 Wash the column three times with deionized water as in the previous step.
Add 500 µl of the Metal solution and incubate for 5 minutes with end-over-end mixing.
Centrifuge 1 minute at 1000 g, discard the flow through.
Wash two more times with deionized 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 purification: wash the column with 500 µl elution buffer, centrifuge the column for 30 - 60 seconds at 1000 g, repeat with 500 µl wash buffer.
If the column was stored before: remove the bottom cap and centrifuge the column for 30 - 60 seconds at 1000 g. Then add 500 µl of wash buffer and centrifuge the column for 30 - 60 seconds at 1000 g.

3 Close the bottom of the column and add 500 µl sanitization solution. Close the lid and incubate for one hour at RT.

4 Remove the bottom cap and centrifuge the column for 30 - 60 seconds at 1000 g.
Wash three times with 500 µl of deionized water.

Storage

5 Close the bottom of the column, add 500 µl 20 % ethanol or wash buffer (contains 0.05 % (w/v) sodium azide). Close the lid and store at room temperature or at 4 - 8 °C.