ChroPlate - IMAC

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ABSTRACT

Purification Guide for the Isolation of Histidine-tagged Proteins with ChroPlate Filtration Plates by DALEX Biotech.

ChroPlate-IMAC by DALEX Biotech offers a robust and convenient way to isolate polyhistidine-tagged proteins 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 plates for analysis, e.g. SDS-PAGE

MATERIALS TEXT

Materials provided in the kit:
ChroPlate
Dummy plate
Wash buffer
Elution buffer
Nickel solution
Cobalt solution

Materials not provided in the kit:
DNase/lysozyme

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Denaturing wash buffer
Denaturing elution buffer

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?
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 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 instructions of your instrument's 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 filter plate.

How do you want to purify?

5 Please choose below how you want to proceed.

step case

Purification by Centrifu

no description provided

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Equilibration

6 Add 200 µl of nickel or cobalt solution to each well. Place the ChroPlate on a deep-well plate and centrifuge at 1000 g for 60 seconds in a swing-out rotor. For counterbalance of the centrifuge a dummy filter plate is included in the kit.

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 inverse 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 Empty the deep-well plate and add 500 µl deionized water to each well of the ChroPlate. Centrifuge at 1000 g for 5 minutes in a swing-out rotor.
Repeat once more.

8 Empty the deep-well plate and add 500 µl wash buffer to each well of the ChroPlate. Centrifuge at 1000 g for 5 minutes in a swing-out rotor.

Load and Wash

9 Place the ChroPlate onto a clean deep-well plate. Add up to 1.5 ml cleared lysate and centrifuge at 1000 g for 5 - 10 minutes in a swing-out rotor.

NOTE

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

Repeat the loading step when your sample volume is larger than 1.5 ml. This is particularly recommended when your target protein has low expression levels.

The centrifugation time depends on the sample's volume and viscosity. Volumes larger than 1 ml and viscous samples e.g. serum usually require more than 5 minutes centrifugation time.

10 Empty the deep-well plate or use a clean one, add 500 µl wash buffer to each well of the ChroPlate, and centrifuge at 1000 g for 5 minutes in a swing-out rotor.
Repeat once more.

NOTE

For an increase in purity repeat this step a third time. However, the yield will be lowered.

Elution

11 Place the ChroPlate on a clean deep-well plate. Add 100 µl elution buffer to each well and centrifuge 5 minutes at 1000 g in a swing-out rotor. Elute two more times into the same deep-well plate.

Equilibration

step case

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Purification by Vacuum

no description provided

6 Assemble your vacuum device according to the manufacturer's instructions.
Add 200 µl nickel or cobalt solution to each well, place the ChroPlate on a waste receptacle and apply a vacuum of 100 mbar for 2 minutes.

NOTE

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

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7 Add 500 µl deionized water to each well, place the ChroPlate on a waste receptacle and apply a vacuum of 100 mbar for 2 minutes.
Repeat once more.

step case

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Repeat once more.

step case

Purification by Vacuum

no description provided

NaN Assemble your vaccum device according to the manufacturer's instructions.
Add 200 µl metal solution to each well, place the ChroPlate on a waste recepticle and apply a vacuum of 100 mBar for 2 minutes.

NOTE

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NaN Add 500 µl water to each well, place the ChroPlate on a waste recepticle and apply a vacuum of 100 mBar for 2 minutes.

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Buffer Preparation

step case

no description provided

NaN **Binding 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 no 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 month. Do not freeze and thaw the buffer more than once.

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

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Sample Preparation

3 Determine the weight of the frozen 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.

Equilibration

step case

Purification by Centrifu

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Repeat once more.

step case

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