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## ChroPlate - IMAC V.2

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Works for me

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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, 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>

### 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  
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) **[M]0.01 Mass / % volume 0.1mg/ml each**  
Alternatively, use ultrasonication according to the instructions of your instrument's manufacturer and skip the next step.



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.



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 Centrifugation

#### Equilibration

- 6 Add 200 µl of nickel or cobalt solution to each well. Place the ChroPlate on a deep-well plate and centrifuge at 400 g for 60 seconds in a swing-out rotor. For counterbalance of the centrifuge a dummy filter plate is included in the kit.



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

The affinity of histidine towards the metal ions is in the order  $\text{Cu} > \text{Ni} > \text{Zn} > \text{Co}$ . 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 400 g for 2 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 400 g for 2 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 400 g for 2 - 10 minutes in a swing-out rotor.



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 400 g for 2 minutes in a swing-out rotor.  
Repeat once more.



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 300 µl elution buffer to each well and centrifuge 2 minutes at 400 g in a swing-out rotor.  
Elute two more times into the same deep-well plate.

#### Equilibration

step case

### Purification by Centrifugation

- NaN 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.



- Apart from  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$ , you can also use  $\text{Cu}^{2+}$  or  $\text{Zn}^{2+}$ .
- The affinity of histidine towards the metal ions is in the order  $\text{Cu} > \text{Ni} > \text{Zn} > \text{Co}$ . 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.

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

step case

### Purification by Vacuum Filtration

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



- Apart from  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$ , you can also use  $\text{Cu}^{2+}$  or  $\text{Zn}^{2+}$ .
- The affinity of histidine towards the metal ions is in the order  $\text{Cu} > \text{Ni} > \text{Zn} > \text{Co}$ . 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.

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

## 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

[M]0.05 Molarity (M) Sodium dihydrogen phosphate

[M]4 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]4 Molarity (M) Urea

[M]0.3 Molarity (M) Sodium chloride

[M]0.250 Molarity (M) Imidazole

Adjust to pH 8.0 with NaOH



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 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) [M]0.01 Mass / % volume 0.1mg/ml each  
Alternatively use ultrasonication according to the instructions of your instrument manufacturer.



Pre-chill an appropriate centrifuge to 4 °C.

## Purification by Centrifugation

NaN Add 200 µl of nickel or cobalt solution to each well. Place the ChroPlate on a deep-well plate and centrifuge at 400 g for 60 seconds in a swing-out rotor. For counterbalance of the centrifuge a dummy filter plate is included in the kit.



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