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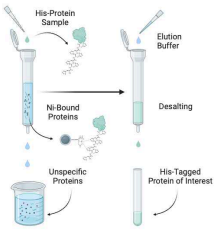
Micro Volume Purification of His-Tagged Proteins with IMAC Ni-Charged Resin



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Syon Schlecht[1,2], Emily Gunderson[2], Ruthie Fowler[1,2], Takara Aguilar[3,2]

[1]Georgia Institute of Technology; [2]Empower College and Career Center; [3]University of Georgia

Syon Schlecht
Georgia Insitute of Technology

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We use this protocol and it's working

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Disclaimer

Abstract

This protocol describes a micro volume purification method for His-tagged proteins using IMAC with Ni-charged resin in a mini spin chromatography column. His-tagged proteins are selective bound to a Ni-charged resin due to the interaction between the polyhistidine tag and Ni^{2+} ions and eluted using imidazole-containing buffers. Desalting is the performed using SEC where salts and imidazole are fractionated, while larger molecules like the target protein are excluded. This results in the isolation of purified His-tagged proteins with a high yield and minimal contaminants, suitable for downstream applications.

Image Attribution

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Protocol materials

- Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories**
- Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories**
- Profinity™ Ni-charged IMAC Resin **Bio-Rad Laboratories** **Catalog #**1560131
- Equilibration Buffer
- Wash Buffer
- Elution Buffer

Preparing and Equilibrating Column

6m

1 Resuspend Profinity™ Ni-charged IMAC Resin **Bio-Rad Laboratories Catalog #**1560131 and pipette 200 µL into a Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories**

2 Centrifuge the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** at 1000 x g, 00:02:00 and discard any collected buffer

2m

3 Wash the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** by adding 200 µL of distilled water

4 Centrifuge the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** at 1000 x g, 00:02:00 and discard any water collected

2m

5 Equilibriate the column by adding 200 µL of Equilibration Buffer to the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories**

Note

The equilibration buffer is 20 mM sodium phosphate, 300 mM NaCl, and 5 mM imidazole.

6 Centrifuge the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** at 1000 x g, 00:02:00 and discard any buffer collected and cap the column

2m

Sample Binding and Elution of His-Proteins

20m

7 From the soluble fraction of lysed cells containing the His-Sample add 600 µL to the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories**

20m

and gently mix for 00:20:00

8 Centrifuge the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** at 1000 x g, 00:02:00 and discard any flowthrough 2m

9 Add 600 µL of Wash Buffer to the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** and centrifuge at 1000 x g, 00:02:00 and discard any collected buffer 2m

Note

The wash buffer is 20 mM sodium phosphate, 300 mM NaCl and 10 mM imidazole.

10 Add 100 µL Elution Buffer to the Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** and centrifuge at 1000 x g, 00:02:00 , and **COLLECT** the flowthrough, **containing** the His-Sample 2m

Note

The elution buffer is 20 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, pH 8.0

Note

The Mini Bio-Spin® Chromatography Column **Bio-Rad Laboratories** can now be disposed of

Desalting of His-Sample 2m

11 Snap the bottom and remove the top of a Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories** and allow the packing buffer to drain for 00:05:00 5m

> Note
>
> The Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories** has a fractionation range of 1000 to 6000 Da, so any remaining salts or small proteins will be fractionated.

12 Centrifuge the Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories** at 1000 x g, 00:02:00 and discard any collected buffer 2m

13 Add the approx. 100 µL of the collected His-Sample to the Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories**

> Note
>
> The maximum load volume of the column is 100 µL

14 Centrifuge the Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories** at 1000 x g, 00:05:00 and any excluded liquid is the purified His-Sample 5m

15 Repeat up to 3 times with the same Micro Bio-Spin® Column with Bio-Gel® P-6 **Bio-Rad Laboratories** to obtain the desired His-Sample volume

Protocol references

Protein Expression and Purification Series.
https://www.bio-rad.com/webroot/web/pdf/lse/literature/pepsi_hr_1665067.pdf

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His-tagged Proteins – Production and Purification. ThermoFisher Scientific.
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