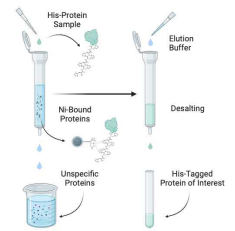


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Micro Volume Purification of His-Tagged Proteins with IMAC Ni-Charged Resin

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Protocol status: Working

We use this protocol and it's working

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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 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
- 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
- 5 Equilibrate 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

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**



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.

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