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His-Spin Protein Miniprep - CHEM 584

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ABSTRACT

The His-Spin Protein Miniprep provides researchers with fast His-tagged protein purification. The simplified procedure is based on an innovative protein purification chemistry and custom-designed fast spin columns. Up to 1 mg of His-tagged protein can be purified in 5 minutes and eluted in as little as 100 µl of His-Elution Buffer. The purified protein can be used directly for enzymatic assays, protein biochemical analyses, SDS-PAGE, and other applications. The product has been optimized for maximal protein purity: a single protein band is visible by Coomassie blue staining on SDS-PAGE gel. The straightforward spin – wash – elute protocol dramatically simplifies protein purification: get results in minutes, not hours.

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GUIDELINES

The procedure can be conducted in cold or at room temperature. Use cold buffers and work on ice for sensitive proteins. Pay attention to centrifugation times: times listed include the time needed for acceleration. Centrifugal steps are carried out in standard tabletop microcentrifuge at maximum speed, usually corresponding to 13,000 to 15,000 g. Read the SAMPLE PREPARATION section below to make sure that the samples are in the correct buffer before loading on the column.

MATERIALS TEXT

Kit Contents:

	P2001 (10 purifications)	P2002 (50 purifications)	Storage*
Zymo-Spin P1 columns	10	50	Room Temperature
Collection tubes	10	50	Room Temperature
His-Affinity Gel	2.8 ml	14 ml	4°C
His-Binding Buffer	10 ml	50 ml	4°C
His-Wash Buffer	10 ml	50 ml	4°C
His-Elution Buffer	5 ml	25 ml	4°C
Instruction sheet	1	1	

* The buffers should be stored refrigerated. However, they may be stored for up to 3 months at room temperature without any effect on stability. Keep the buffers cold, or put them on ice before use for purification of sensitive proteins. The affinity gel may be kept at room temperature for up to 7 days.

Composition of Supplied Solutions

His-Binding buffer
50 mM sodium phosphate buffer pH 7.7
300 mM sodium chloride
10 mM imidazole
0.03 % Triton X-100

His-Wash buffer
50 mM sodium phosphate buffer pH 7.7
300 mM sodium chloride
50 mM imidazole
0.03 % Triton X-100

His-Elution buffer
50 mM sodium phosphate buffer pH 7.7
300 mM sodium chloride
250 mM imidazole

His-Affinity gel
Nickel-charged agarose – 30 % by volume
20 mM sodium phosphate buffer pH 7.7
100 mM sodium chloride
10 mM imidazole
ethanol – 20 % by volume

SAFETY WARNINGS

Consult SDS sheets for specific safety warnings.

His-Spin Protein Miniprep

1 Transfer **250 µl** of His-Affinity Gel to the Zymo-Spin P1 column (make sure the resin is fully resuspended by shaking/vortexing the bottle before pipetting) and place the column into a collection tube.

Use a 1 ml pipette tip to transfer the His-Affinity Gel.; a 200 µl-size (usually yellow) or smaller automatic pipette tip has a small opening and may not be large enough for the affinity gel particles.

2 Centrifuge for 5-10 seconds.

Ensure that the His-Affinity Gel is completely drained. Some centrifuge models may require longer centrifugation times. Do not over-dry the gel by long centrifugation times.

3 Add **100 µl** to **300 µl** of your protein sample and resuspend the gel by shaking or tapping the column.

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Resuspend the gel a few more times during a **00:02:00** incubation period.

It is important to allow the gel and your sample to interact for at least two minutes. If the sample volume is larger than 200 µl, an additional 1-2 minutes binding time may be needed to improve yields of purified protein.

4 Centrifuge the column/collection tube 5-10 seconds. Discard the flow-through and place the column back in the collection tube.

5 Add **250 µl** of His-Wash Buffer and resuspend the gel. Centrifuge 5-10 seconds.

6 Repeat the above wash step (step #5) one more time. Discard the collection tube.

7 Place the Zymo-Spin P1 column into a standard microcentrifuge tube. Add **150 µl** of His-Elution Buffer to the column and resuspend the gel.

Elution volumes can be between 100-200 µl. 150 µl of His-Elution Buffer elutes virtually all the column-bound protein. Smaller elution volumes are also possible and may yield more concentrated protein, but the elution efficiency may be compromised.

8 Centrifuge 5-10 seconds to elute the purified protein.

The eluate now contains the purified protein. The eluted protein is suitable for many applications. Use 1-10 µl for SDS-PAGE and Coomassie blue staining analysis. Store the purified protein at an appropriate temperature.

OTHER TECHNICAL CONSIDERATIONS

9 **1. Starting material containing incompatible components such as EDTA, EGTA, DTT, > 15 mM β-mercaptoethanol, > 10 mM imidazole or histidine.**
If your starting material contains these compounds, dilution with the His-Binding Buffer may help. Multiple loadings on the column will be necessary to load enough material. If the sample is in a different buffer, adjust the pH and imidazole and salt concentrations, and carry out a test preparation. If the protein is still not bound, the sample needs to be dialyzed before use.

2. Diluted starting material
If your starting material contains low levels of His-tagged protein and it requires more than 300 ul starting sample to purify enough protein, repeat steps #3 and #4 of the Protocol by loading 300 ul sample each time to mix with the His-Affinity Gel.

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3. No purified protein recovered

There are several possible explanations for recovering no protein. Often, the His-tag may be rendered inaccessible as a result of protein folding. The recombinant protein can also be insoluble as a result of overexpression. In both cases, the protein can be purified at denaturing conditions (see below). On rare occasions, the protein is bound to the His-Affinity Gel too tightly and can not be eluted with the supplied His-Elution Buffer. A custom-made elution buffer containing 500 mM imidazole or 100 mM EDTA may elute the tightly-bound protein. Also, check your DNA construct for errors.

4. Low yield of purified protein

Protein folding may hinder the binding of the hexahistidine tag to the His-Affinity Gel. In this case, unbound protein is found in the flow-through or wash fractions. Lowering the imidazole concentration of the His-Wash Buffer to 25 mM (e.g., by dilution with His-Binding Buffer) may increase yields. Alternatively, in rare cases, the protein is bound to the His-Affinity Gel too tightly and can not be completely eluted with the supplied His-Elution Buffer. A custom-made elution buffer containing 500 mM imidazole or 100 mM EDTA will help to solve this problem.

5. Eluted protein is not pure

Check your buffers for signs of contamination, and check the pH of the buffers. Also, make sure that centrifugation drains the His-Affinity Gel completely after each spin (some older centrifuge models may require longer centrifugation time). If the problem persists, add an additional wash step in the purification protocol, or increase the imidazole concentration of the washing buffer to 60 – 100 mM (e.g., by dilution with the His-Elution Buffer).

6. Insoluble protein.

Overexpression of proteins may result in the formation of insoluble inclusion bodies inside cells. If a large band of over-expressed protein is visible after SDS-PAGE electrophoresis of whole cells, but the band is absent after SDS-PAGE electrophoresis of cleared cell lysates, this indicates that the protein may not be soluble and the expressed protein may form inclusion bodies.
Such proteins will not be purified using the provided buffers. It is, however, possible to purify such proteins at denaturing conditions in the presence of 8 M urea or 6 M guanidine hydrochloride. The protein native structure and thus enzyme activity is lost under such conditions but may be restored by refolding the protein after purification.
For purification at denaturing conditions, lyse the cells or resuspend inclusion bodies in the denaturing binding buffer (see below). Follow the purification steps described above, replacing the buffers with denaturing buffers.

Denaturing Buffers (not supplied)

Important: Urea decomposition in these buffers may shift pH upon storage – check and re-adjust before use. For improved stability, it is recommended to store these buffers at 4°C or make fresh.
Binding buffer: 8 M urea, 10 mM imidazole, 0.1 M sodium phosphate monobasic, 0.01 M Tris, pH 8.0. Adjust the pH by the addition of concentrated sodium hydroxide.
Washing buffer: 8 M urea, 50 mM imidazole, 0.1 M sodium phosphate monobasic, 0.01 M Tris, pH 6.3. After dissolving the components the solution will be approximately pH 6.3.
Elution buffer: 8 M urea, 250 mM imidazole, 0.1 M sodium phosphate monobasic, 0.01 M Tris, pH 4.5. Adjust the pH by the addition of concentrated hydrochloric acid.

7. Membrane-associated protein

Membrane proteins can be purified after solubilization in a nonionic detergent. Concentrations of up to 2% of Triton® or TWEEN® can be present in the loaded sample.

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