His SpinTrap

Caution! *Contains nickel, may produce an allergic reaction.*

His SpinTrap™ is a prepacked, single-use spin column for rapid purification and screening of histidine-tagged proteins by immobilized metal affinity chromatography (IMAC). The column can be used with a standard microcentrifuge, and one purification run takes approximately 10 min.

His SpinTrap contains Ni Sepharose™ High Performance medium, which has high protein binding capacity, low nickel ion (Ni^{2+}) leakage, and excellent compatibility with denaturing agents plus a wide range of additives. Both clarified and unclarified samples can be applied. Table 1 lists the main characteristics of His SpinTrap.

Table 1. His SpinTrap characteristics

Column material	Polypropylene barrel, polyethylene frits
Medium	Ni Sepharose High Performance
Average bead size	34 µm
Protein binding capacity[1]	Approx. 750 µg histidine-tagged protein/column
Bed volume	100 µl
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents. (See Table 2.)
Avoid in buffers	Chelating agents, e.g. EDTA, EGTA, citrate. (See Table 2.)
Storage	0.15% Kathon™ CG
Storage temperature	+4 to +30°C

[1] Binding capacity is protein-dependent.

Recommended buffers

Recommended buffers for native conditions can easily be prepared from His Buffer Kit (Code No. 11-0034-00) or according to the description in Appendix A.

Native conditions:

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

Denaturing conditions:

Binding buffer: 20 mM Tris-HCl, 8 M urea, 500 mM NaCl, 5 mM imidazole, pH 8.0 + 1 mM β-mercaptoethanol

Elution buffer: 20 mM Tris-HCl, 8 M urea, 500 mM NaCl, 500 mM imidazole, pH 8.0 + 1 mM β-mercaptoethanol

The optimal concentration of imidazole needed in the sample and buffer to obtain the best purity and yield differs from protein to protein. Under native conditions, 20–40 mM imidazole in the binding buffer is suitable for many proteins. 500 mM imidazole in the elution buffer is most often sufficient to completely elute the target protein.

Note: As an alternative to elution with imidazole, you can lower the pH to approx. pH 4.5 (note that metal ions will be stripped off the medium below pH 4.0).

Sample preparation

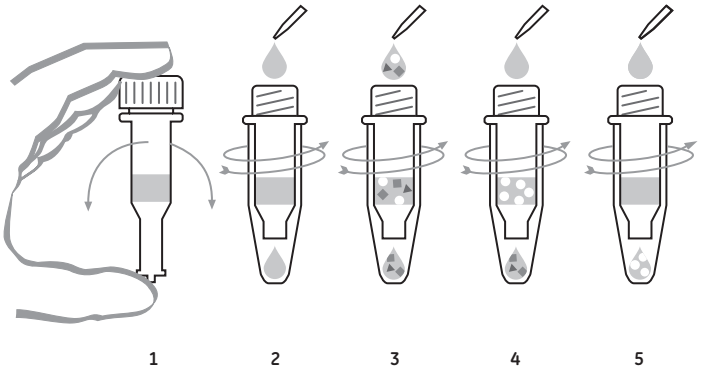
The protocol below has been used successfully in our own laboratories, but other established procedures may also work. Use standard 2-ml microcentrifuge tubes.

1. **Dilute the cell paste:** Add 1 ml binding buffer to resuspend cell paste obtained from 20–50 ml cell culture (depending on expression level). To prevent host cell proteins binding to exposed histidines, it is essential that the sample and binding buffers contain the same concentration of imidazole.
2. **Enzymatic lysis:** Add the following substances to final concentrations in the cell suspensions: 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF. Vortex the tubes gently and incubate at room temperature for 30 min.

   Chemical lysis kits can also be used, but make sure that they do not contain any chelating agent.
3. **Mechanical lysis:** Repeated freeze/thaw or sonication.
4. **Clarify the lysate:** Spin at full speed in a microcentrifuge for 10 min to remove insoluble material. Collect supernatants and purify on His SpinTrap.

Note: You can also apply unclarified sample to the column (i.e. omit centrifugation in step 4 above).

Performing a purification



Run purifications on His SpinTrap using a standard microcentrifuge. Place the column in a 2-ml microcentrifuge tube to collect the liquid during centrifugation. Use a new 2-ml tube for every step (steps 1-5).

1. Invert and shake the column repeatedly to resuspend the medium. Loosen the top cap one-quarter of a turn and break off the bottom closure. Place the column in a 2-ml microcentrifuge tube and centrifuge for 30 s at 70 × g (approx. 1000 rpm in an Eppendorf™ 5415R, 24 position fixed-angle rotor) to remove the storage liquid.
2. Remove and discard the top cap. Equilibrate the column by adding 600 µl binding buffer. Centrifuge for 30 s at 70 × g.
3. Add the sample (see Sample preparation). Maximum sample volume is 600 µl in one go. Centrifuge for 30 s at 70 × g. You can make several sample applications as long as you do not exceed the capacity of the column.
4. Wash with 600 µl binding buffer. Centrifuge for 30 s at 70 × g.
5. Elute the target protein twice with 200 µl elution buffer. Centrifuge for 30 s at 70 × g and collect the purified sample. The first 200 µl will contain the majority of the target protein.

Table 2. His SpinTrap is compatible with the following compounds at the concentrations given.

Reducing agents	5 mM DTE 5 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione
Denaturing agents*	8 M urea 6 M guanidine-HCl
Detergents	2% Triton™ X-100 (nonionic) 2% Tween™ 20 (nonionic) 2% NP-40 (nonionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA† 60 mM citrate†
Buffers	50 mM sodium phosphate, pH 7.4 100 mM Tris-HCl, pH 7.4 100 mM Tris-acetate, pH 7.4 100 mM HEPES, pH 7.4 100 mM MOPS, pH 7.4 100 mM sodium acetate, pH 4*

* Tested for one week at +40°C.

† Generally, chelating agents should be used with caution (and only in the sample, not in the buffers). Any metal-ion stripping may be counteracted by adding a small excess of $MgCl_2$ before centrifugation/filtration of the sample.

Troubleshooting

Fault	Possible cause	Action
Liquid not completely removed during centrifugation	Sample too viscous	Increase centrifugation time. Increase dilution of the cell paste before or after mechanical lysis. Continue mechanical lysis until the viscosity is reduced, and/or add an additional dose of DNAse and Mg^{2+}. Filter the sample (or centrifuge if you have used unclarified sample).
	Target protein difficult to dissolve or precipitates during purification	Add detergents, reducing agents or other additives (see Table 2) and mix gently for 30 min to aid solubilization of the tagged protein. Note that Triton X-100 and NP-40 (but not Tween) have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange. Inclusion bodies: the protein can usually be solubilized (and unfolded) from inclusion bodies using common denaturants such as 4–6 M guanidine-HCl, 4–8 M urea or strong detergents. Mix gently for 30 min or more to aid solubilization.
Low yield of histidine-tagged protein	Histidine-tagged protein found in the flowthrough during sample application and wash	Imidazole concentration in the sample and binding buffer is too high. Use a lower concentration. Ensure that the concentration of chelating or strong reducing agents in the sample is not too high. The histidine tag may be insufficiently exposed; perform purification of unfolded protein in urea or guanidine-HCl as for inclusion bodies. To minimize dilution of the sample, add solid urea or guanidine-HCl. The histidine tag has been lost. Check the sequence of the construct.
	Histidine-tagged protein not eluted during purification	Histidine-tagged protein still bound. Elute with a higher concentration of imidazole in the elution buffer. The target protein has precipitated in the column. Decrease the amount of sample. Decrease imidazole concentration during elution. Try detergents or change NaCl concentration, or elute under denaturing (unfolding) conditions. Nonspecific hydrophobic or other interaction. Add a nonionic detergent to the elution buffer or increase NaCl concentration.
	Histidine-tagged protein not completely eluted	Elute with a larger volume of elution buffer.
Eluted histidine-tagged protein not pure	Imidazole concentration in sample and binding buffer too low	Increase imidazole concentration in sample and binding buffer to prevent contaminants binding. We recommend 20–40 mM, but higher concentrations may also work.
	Partial degradation of tagged protein by proteases	Add protease inhibitors (use EDTA with caution, see Table 2). Perform lysis and purify at 4°C.
	Contaminants are associated with tagged proteins	Add detergent and/or reducing agents before sonicating the cells. Increase detergent levels (e.g. up to 2% Triton X-100 or 2% Tween), or add glycerol (up to 50%) to the wash buffer to disrupt nonspecific interactions.
	Insufficient washing of unbound material	Repeat the wash step after sample application to obtain optimal purity.

Ordering information

Designation	No. supplied	Code No.
His SpinTrap	50	28-4013-53

Related products	No. supplied	Code No.
His Buffer Kit	1	11-0034-00
(contains 2 × 100 ml phosphate buffer, 8x stock solution, pH 7.4 and 1 × 100 ml 2 M imidazole, pH 7.4)		

Literature	Code No.
Recombinant Protein Handbook, Protein Amplification and Simple Purification	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86
Data File Ni Sepharose High Performance	18-1174-40

Appendix A

2 M imidazole stock solution

To 34.05 g imidazole, add distilled water to 200 ml and dissolve completely. Adjust to pH 7.4 with HCl. Add distilled water to 250 ml. Use high purity imidazole as this will give no or very low absorbance at 280 nm (imidazole, 68.08 g/mol).

Phosphate buffer (containing imidazole for binding and elution buffers)

(20 mM sodium phosphate, 500 mM NaCl, 10–500 mM imidazole in 250 ml)

To 0.44 g $Na_2HPO_4 \times 2H_2O$ (177.99 g/mol), 0.35 g $NaH_2PO_4 \times H_2O$ (137.99 g/mol) and 7.30 g NaCl (58.44 g/mol), add X ml (see table below) 2 M imidazole stock solution. The volume of imidazole stock solution added depends on the chosen imidazole binding and elution concentrations. Finally, add distilled water to 200 ml and dissolve completely.

Adjust to pH 7.4 with HCl. Add distilled water to 250 ml and filter through a 0.45-µm filter.

Imidazole concentration in buffer (mM)	Volume of imidazole stock solution in phosphate buffer (ml)
10	1.25
20	2.5
30	3.75
40	5
50	6.25
60	7.5
70	8.75
80	10
90	11.25
100	12.5
200	25
300	37.5
400	50
500	62.5

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