His **SpinTrap**
His **SpinTrap** Kit

TAGGED PROTEIN PURIFICATION

His SpinTrap™ is a prepacked, single-use spin column for purifying histidine-tagged proteins by immobilized metal ion affinity chromatography (IMAC). The column allows fast and simple small-scale purification and is a valuable tool for screening purposes and high-throughput applications. His SpinTrap is used with a standard microcentrifuge and one purification run takes approx. 10 min.

His SpinTrap Kit (Fig 1) also includes His Buffer Kit to promote fast, reproducible and convenient purification work.

His SpinTrap is a member of the Trap platform, which addresses the need for flexible, small-scale preparation of samples before downstream analyses such as gel electrophoresis, liquid chromatography and LC-MS.

His SpinTrap allows:

- High protein binding capacity — up to 750 µg pure histidine-tagged protein per column
- Direct purification of unclarified, as well as clarified cell lysates
- Short purification times — approx. 10 min per run

His SpinTrap columns contain Ni Sepharose™ High Performance, which has negligible nickel leakage and is compatible with denaturing and reducing agents, as well as a wide range of additives.

Table 1 lists the main characteristics of the column.

Operation

The purification of histidine-tagged proteins on His SpinTrap can be divided into four stages: equilibration, sample application, washing, and elution (Fig 2). Each step involves centrifugation using a microcentrifuge.

Fig 1. His SpinTrap columns are designed for efficient, small-scale purification of histidine-tagged proteins directly from clarified or unclarified cell lysates. His Buffer Kit adds convenience and reproducibility.

Table 1. His SpinTrap characteristics

Column material	Polypropylene barrel, polyethylene frits
Medium	Ni Sepharose High Performance
Medium volume	100 µL
Average bead size	34 µm
Protein binding capacity[1]	Approx. 750 µg histidine-tagged protein/column
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°C to 30°C

[1] Binding capacity is protein-dependent.

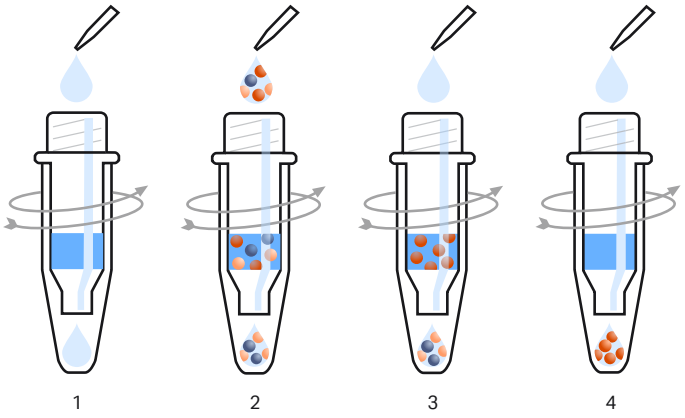


Fig 2. Purifying histidine-tagged proteins with His SpinTrap is a simple procedure in four stages, all involving brief centrifugation on a microcentrifuge. The entire process takes approximately 10 minutes: (1) after placing the column in a 2 mL microcentrifuge tube, equilibrate by adding binding buffer and centrifuge; (2) add sample; (3) wash with binding buffer; (4) elute the target protein with elution buffer.

The concentration of imidazole in the sample and binding buffers influences final product purity. Imidazole must be present in these buffers, and the optimal concentration range for His SpinTrap is 20-40 mM. Elution is simply performed either by using elution buffer containing 500 mM imidazole or by reducing the pH of the binding buffer to 4.5. Purification can be performed either under native or denaturing conditions, and a number of alternative additives can be used.

His SpinTrap Kit

For optimal performance and convenience use His SpinTrap Kit, consisting of His SpinTrap and His Buffer Kit (Fig 1). His Buffer Kit provides phosphate buffer concentrates and highly pure 2 M imidazole stock solutions, which saves time and improves reproducibility.

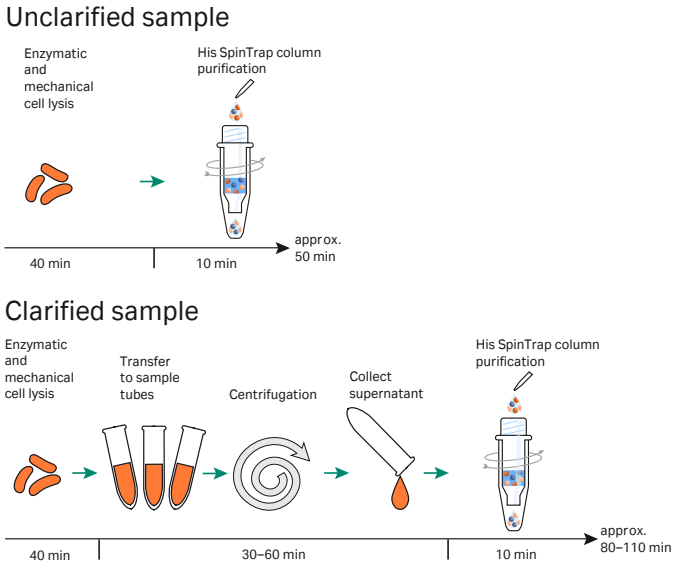


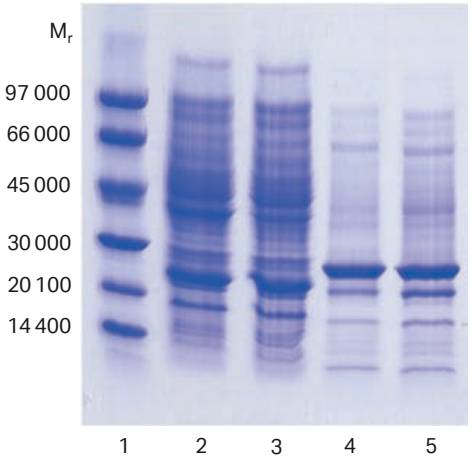
Fig 3. By eliminating the need to clarify cell lysates by centrifugation, the total time required for preparing and purifying unclarified samples is reduced by 30–60 min.

Purification of unclarified sample

His SpinTrap columns allow direct purification of unclarified cell lysates. The recommended procedure for preparing samples is enzymatic lysis followed by mechanical lysis, for example, sonication. Purifying unclarified samples saves time by eliminating centrifugation, which normally takes 30–60 min, including tube handling (Fig 3). The risk of losing target protein during manual operations such as transfer to centrifugation tubes and collecting supernatant is also eliminated. In addition, the shorter sample preparation time minimizes degradation and oxidation of sensitive target proteins.

The performance of His SpinTrap columns in purifying a histidine-tagged protein from unclarified *E. coli* lysate has been assessed. Lysate from *E. coli* BL-21 containing GFP-$(His)_6$ was subjected to enzymatic lysis followed by sonication for 10 min and the unclarified lysate was loaded directly on His SpinTrap. For comparison, half of the sample was clarified by centrifugation before loading. Samples and binding buffer contained 60 mM imidazole. To ensure complete elution of GFP-$(His)_6$, which has a high affinity for Ni Sepharose High Performance, the elution buffer contained 800 mM imidazole rather than the normally recommended concentration of 500 mM.

Column:	His SpinTrap
Equilibration:	600 µL binding buffer
Sample application:	600 µL unclarified or clarified *E. coli* BL-21 lysate containing 150 µg GFP-$(His)_6$
Wash:	600 µL binding buffer
Elution:	2 × 200 µL elution buffer
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 60 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 800 mM imidazole, pH 7.4



Lanes
1. LMW markers
2. Unclarified sample, start material (diluted 1:10)
3. Clarified sample, start material (diluted 1:10)
4. Unclarified sample, eluted pool
5. Clarified sample, eluted pool

Fig 4. SDS-PAGE (ExcelGel™ SDS Gradient 8–18) of unclarified and clarified lysate from *E. coli* with GFP-$(His)_6$. Similar purity and recovery were observed for both unclarified and clarified samples. The gel was run under reducing conditions.

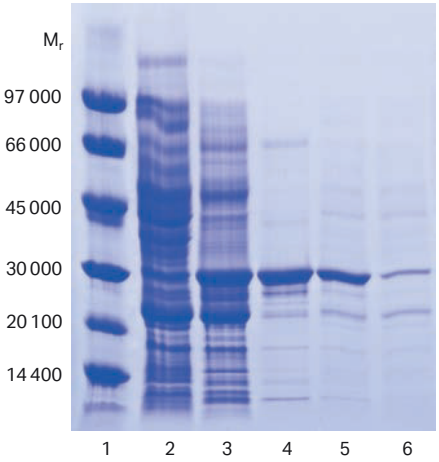
Purification time for the unclarified and clarified samples was 10 min. The final purity of the eluates from the samples were similar as confirmed by SDS-PAGE (Fig 4).

Optimizing purification conditions

The imidazole concentration during binding and washing is an important factor affecting the final purity and yield of the target protein. This was demonstrated by a series of experiments where a histidine-tagged protein, APB 7-$[His]_6$ (M_r 28 000), was purified on His SpinTrap using 5, 50, 100, or 200 mM imidazole in samples and binding buffers. The elution buffer contained 500 mM imidazole.

An imidazole concentration of 5 mM resulted in low purity of the eluted sample, while an increase to 50 mM imidazole prevented binding of contaminants and improved purity (Fig 5, lane 4). The presence of 100 mM imidazole in the sample and binding buffers lowered recovery while purity improved marginally (Fig 5, lane 5). The lower recovery can be explained by leakage of target protein due to the high imidazole concentration during binding and washing. A further increase to 200 mM imidazole reduced recovery even more (Fig 5, lane 6).

Column:	His SpinTrap
Equilibration:	600 µL binding buffer
Sample application:	600 µL clarified *E. coli* BL-21 lysate containing 400 µg APB 7-$(His)_6$
Wash:	600 µL binding buffer
Elution:	2 × 200 µL elution buffer
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 5–200 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



Lanes
1. LMW markers
2. Start material (diluted 1:10)
3. Eluted pool, 5 mM imidazole during binding (diluted 1:2)
4. Eluted pool, 50 mM imidazole during binding (diluted 1:2)
5. Eluted pool, 100 mM imidazole during binding (diluted 1:2)
6. Eluted pool, 200 mM imidazole during binding (diluted 1:2)

Fig 5. SDS-PAGE (ExcelGel SDS Gradient 8-18) of histidine-tagged APB 7 protein. The imidazole concentration during binding affects the final purity and recovery (compare lanes 3, 4, 5, and 6). The gel was run under reducing conditions.

In summary, higher imidazole concentrations during binding improve the purity, whereas too high a concentration decreases the recovery. The imidazole concentration required for optimal binding is protein-dependent and must be empirically determined. For many proteins, however, 20–40 mM imidazole is a good starting concentration.

Use
20-40 mM Imidazole
in sample and binding buffer
FOR HIGHEST PURITY

High protein binding capacity

His SpinTrap columns are delivered pre-packed with Ni Sepharose High Performance medium, which has a high capacity for protein binding. This has been demonstrated by loading increasing amounts (40–1350 µg) of pure histidine-tagged maltose binding protein (MBP-$[His]_6$) on His SpinTrap. The sample and binding buffers contained 5 mM imidazole and the elution buffer contained 500 mM imidazole. Recovery was calculated using the extinction coefficient and absorbance measurements.

Sample recovery was 80–100% from loads of up to approximately 1000 µg MBP-$(His)_6$ (Fig 6).

Column:	His SpinTrap
Equilibration:	600 µL binding buffer
Sample application:	600 µL pure MBP-$(His)_6$
Wash:	600 µL binding buffer
Elution:	2 × 200 µL elution buffer
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

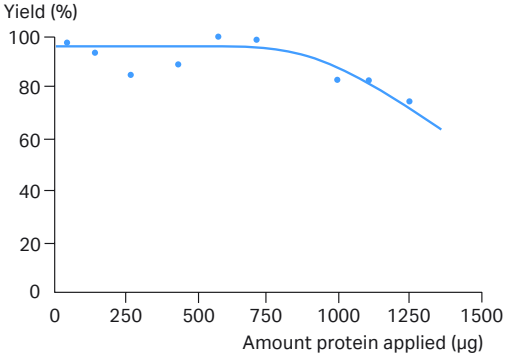


Fig 6. The high protein binding capacity of His SpinTrap gives high sample recovery over a wide range of loads.

High stability and compatibility

His SpinTrap contains Ni Sepharose High Performance medium, which consists of 34 µm beads of highly crosslinked agarose to which a chelating ligand has been immobilized and charged with Ni^{2+} ions. The medium is compatible with a wide range of additives commonly used when purifying histidine-tagged proteins (see Table 2).

Table 2. His SpinTrap spin columns are 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). Metal-ion stripping may be counteracted by adding a small excess of $MgCl_2$ before sample centrifugation/filtration.

Acknowledgement

GFP-$(His)_6$ was provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden. MBP-$(His)_6$ was provided by Pharmacia Diagnostics, Uppsala, Sweden. The plasmid expressing the APB 7 protein was kindly donated by SGX Pharmaceuticals Inc., San Diego, USA.

Ordering information

Product	Quantity	Code No.
His SpinTrap	50 × 100 µL	28-4013-53
His SpinTrap Kit[1]	1	28-9321-71
Related product		
His Buffer Kit	1	11-0034-00
(Includes 2 × 100 mL phosphate buffer, 8× stock solution, pH 7.4 and 1 × 100 mL 2 M imidazole, pH 7.4)		

[1] Includes 1 pack His SpinTrap and 1 pack His Buffer Kit.

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