His SpinTrap

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 purifications 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 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 1). Each step involves centrifugation using a microcentrifuge.

The imidazole concentration in the sample and binding buffer influences final product purity. For His SpinTrap, we recommend 20–40 mM imidazole, which is higher than for similar products on the market. Elution is simply performed with elution buffer containing 500 mM imidazole. Lowering to pH 4.5 is an alternative for elution. Purification can be performed either under native or denaturing conditions, and a number of additives can be used.

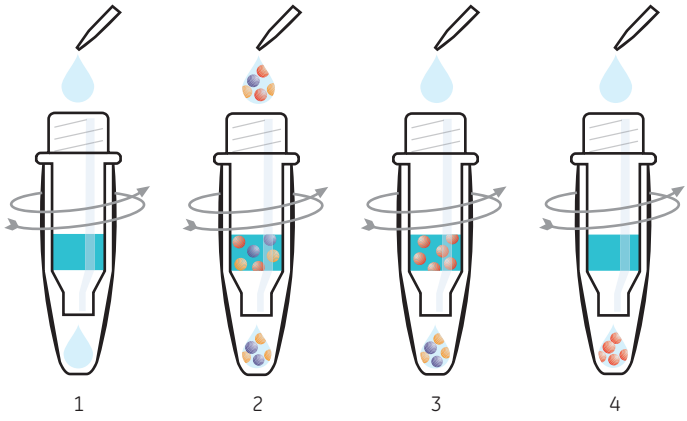


Fig 1. Purifying histidine-tagged proteins with His SpinTrap is a simple, four-stage procedure that can be performed in 10 min using a microcentrifuge: (1) After placing the column in 2-ml microcentrifuge tube, equilibrate by adding binding buffer and centrifuge; (2) add sample; (3) wash with binding buffer; (4) elute the target protein with elution buffer.

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 to +30ºC

[1] Binding capacity is protein-dependent.

Fig 2. His SpinTrap columns are designed for efficient, small-scale purification of histidine-tagged proteins directly from clarified or unclarified cell lysates.

Purification of unclarified sample

His SpinTrap columns allow direct purification of unclarified cell lysates. The proposed 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, etc. (Fig 3). Avoiding centrifugation also reduces the risk of losing target protein during manual operations such as transfer to centrifugation tubes and collecting supernatant. The short sample preparation time generally minimizes degradation and oxidation of sensitive target proteins.

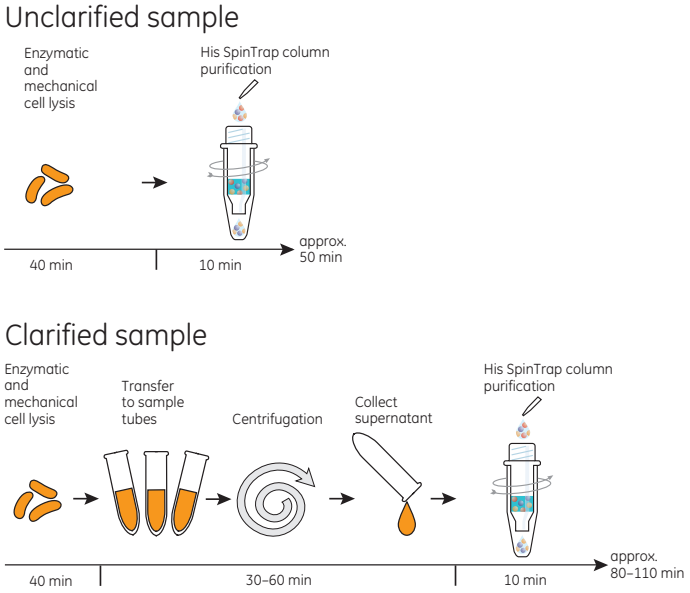


Fig 3. Total times for preparing and purifying unclarified samples are 30–60 min less than for clarified samples as the extra time needed to clarify the cell lysate by centrifugation is eliminated.

The performance of His SpinTrap columns in purifying a histidine-tagged protein from unclarified *E. coli* lysate was assessed. Histidine-tagged green fluorescent protein, GFP-$(His)_6$, in *E. coli* BL-21 lysate 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 also clarified by centrifugation before purification. 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 more usual 500 mM.

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

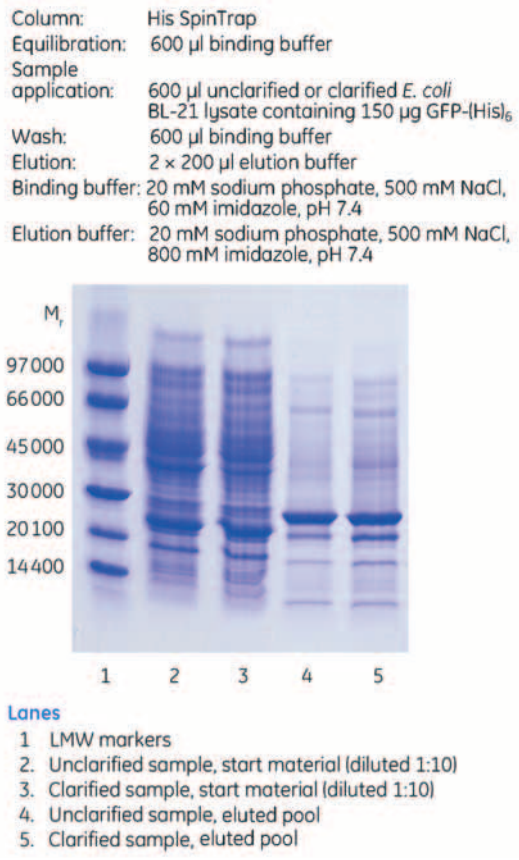


Fig 4. SDS-PAGE (ExcelGel™ SDS Gradient 8–18) under reducing conditions of unclarified and clarified *E. coli* lysate containing GFP-$(His)_6$. Similar purity and recovery was observed for both unclarified and clarified sample.

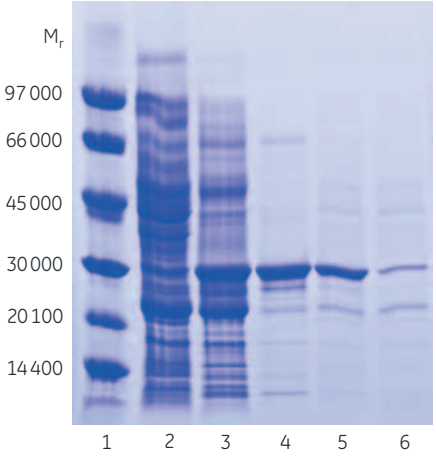
Optimizing purification conditions

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). Including 100 mM imidazole in the sample and binding buffer lowered yield while purity improved marginally (Fig 5, lane 5). The lower yield can be explained by leakage of target protein due to the high imidazole concentration during binding and washing. Further increase to 200 mM imidazole reduced yield even more (Fig 5, lane 6).

In summary, higher imidazole concentrations during binding improve the purity, whereas too high a concentration decreases the yield. The optimal imidazole concentration during binding is protein-dependent. For many proteins, 20–40 mM imidazole is the best choice.

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

High protein binding capacity

His SpinTrap columns are delivered prepacked with Ni Sepharose High Performance, which displays high protein binding capacity. To demonstrate this feature, increasing amounts (40–1350 µg) of pure histidine-tagged maltose-binding protein (MBP-$[His]_6$) were loaded on His SpinTrap. Imidazole concentration in sample and binding buffer was 5 mM and the elution buffer contained 500 mM imidazole. Recovery was calculated using the extinction coefficient and absorbance measurements.

Results showed that high yields (80–100%) were obtained over a broad sample loading interval of up to approximately 1000 µg MBP-$(His)_6$ applied to the column (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

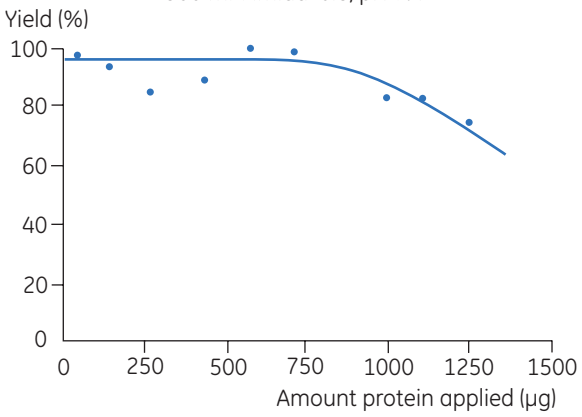


Fig. 6. The high protein binding capacity of His SpinTrap results in high yields over a wide range of applied protein sample loads.

High stability and compatibility

His SpinTrap contains Ni Sepharose High Performance, which consists of 34-µm beads of highly cross-linked 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. Table 2 lists the compatibility of Ni Sepharose High Performance with additives commonly used in the purification of histidine-tagged proteins.

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 ß-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
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)		

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