cytiva

His **SpinTrap**

Instructions

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11003691 AF

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1 Introduction

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

WARNING
The column contains nickel, Ni2+, which is potentially allergenic. Always use normal personal protection devices like gloves and safety glasses when handling His SpinTrap™ columns.

WARNING
The column storage solution, 0.15% Kathon™ CG/ICP Biocide, is potentially allergenic. Use gloves when discarding the storage solution.

Storage.

Store at 4°C to 30°C.

Purpose

His SpinTrap columns are designed for rapid small-scale purification of histidine-tagged proteins. The columns are suitable for purification of multiple samples in parallel, for example in screening experiments. For increased convenience a combination kit is also available consisting of His SpinTrap and His Buffer kit.

His SpinTrap contains:

- 50 prepacked His SpinTrap columns
- Instructions for use

2 Principle

His SpinTrap contains Sepharose™ High Performance medium, which has high binding capacity for histidine-tagged proteins. *Table 1, on page 4* summarizes His SpinTrap characteristics.

Table 1. His SpinTrap characteristics

Column material	Polypropylene barrel, polyethylene frits
Medium	SepharoseHigh 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, on page 6*)
Avoid in buffers	Chelating agents, e.g. EDTA, EGTA, citrate. (See *Table 2, on page 6*)

Storage	0.15% Kathon™ CG
Storage temperature	4°C to 30°C

[1] Binding capacity is protein-dependent.

The column has low nickel ion (Ni^{2+}) leakage and is compatible with denaturing agents and a wide range of additives, see *Table 2, on page 6*.

Table 2. His SpinTrap compatibility.

Reducing agents	5 mM DTE
	5 mM DTT
	20 mM ß-mercaptoethanol
	5 mM TCEP
	10 mM reduced glutathione
Denaturing agents[1]	8 M urea
	6 M guanidine-HCl
Detergents	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[2]
	60 mM citrate[2]
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[1], pH 4

[1] Tested for one week at 40°C.

[2] 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.

His SpinTrap can be used with a standard microcentrifuge, and a purification takes approximately 10 minutes.

Cell culture lysates may be directly applied to the column without prior clarification.

3 Advice on handling

Buffers for native conditions

Recommended buffers for native conditions can easily be prepared from His Buffer Kit or according to the description in Section *Chapter 4 Sample pretreatment protocol, on page 8*.

- 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

Buffers for 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

Optimal imidazole concentration in binding and elution buffers

Binding buffer

The optimal imidazole concentration in the binding buffer is protein dependent and has influence on final yield and purity of the histidinetagged protein. Under native conditions, 20 to 40 mM imidazole in the binding buffer is suitable for many proteins.

Elution buffer

A concentration of 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, pH can be lowered to approximately pH 4.5. At pH below 4.0 the metal ions will be stripped off the medium.*

4 Sample pretreatment protocol

This is the recommended sample pretreatment protocol. However, other established sample pretreatment procedures may also work.

Use standard 2 mL microcentrifuge tubes.

Step	Action
1	Dilute the cell paste **a.** Add 1 mL binding buffer to resuspend cell paste obtained from 20 to 50 mL cell culture (volume depending on expression level). ***Note:*** *To prevent binding of host cell proteins it is essential that the sample and binding buffers contain the same concentration of imidazole.*
2	Enzymatic lysis

Step	Action
	a. Add the following substances to specified final concentrations in the cell suspensions: • Lysozyme: 0.2 mg/mL • DNAse: 20 µg/mL • $MgCl_2$: 1 mM • Pefabloc™ SC or PMSF: 1 mM
	b. Vortex the tubes gently and incubate at room temperature for 30 min. ***Note:*** *Chemical lysis kits can also be used, but make sure that they do not contain any chelating agent.*
3	Mechanical lysis
	a. Repeated freeze/thaw or sonication.
4	Clarify the lysate
	a. Spin at full speed in a microcentrifuge for 10 min to removeinsoluble material.
	b. Collect supernatants and purify on His SpinTrap.
	Note: *Cell culture lysates may be directly applied to the column without prior clarification (i.e. omit step 4).*

5 Purification protocol

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.

Step	Action
1	Remove storage solution
	a. Invert and shake the column repeatedly to resuspend the medium.
	b. Loosen the top cap one-quarter of a turn and twist off the bottom closure.
	c. Place the column in a 2 mL microcentrifuge tube and centrifuge for 30 s at 70 to 100 × g.
	d. Remove and discard the top cap.
2	Column equilibration
	a. Add 600 µL binding buffer.
	b. Centrifuge for 30 s at 70 to 100 × g
3	Sample application
	a. Add up to 600 µL sample in one application.
	b. Centrifuge for 30 s at 70 to 100 × g.
	Note: *Several sample applications can be performed as long as the capacity of the column is not exceeded.*
4	Wash
	a. Add 600 µL binding buffer.

Step	Action
	b. Centrifuge for 30 s at 70 to 100 × g.
5	Elution
	a. Add 200 µL elution buffer.
	b. Centrifuge for 30 s at 70 to 100 × g and collect the purified sample.
	c. Add 200 µL elution buffer.
	d. Centrifuge for 30 s at 70 to 100 × g and collect the purified sample.
	Note: *The first eluted 200 µL will contain the majority of the target protein.*

6 Prepare buffers with different imidazole concentrations

2 M imidazole stock solution

To prepare binding and elution buffer with different amounts of imidazole a 2 M imidazole stock solution should be used. Prepare 250 mL 2 M imidazole stock solution:

Step	Action
1	To 34.05 g imidazole, add distilled water to 200 mL and dissolve completely. Use high purity imidazole as this will give no or very low absorbance at 280 nm (imidazole, 68.08 g/mol).

Step	Action
2	Adjust to pH 7.4 with HCl.
3	Add distilled water to 250 mL.

Binding or elution buffers

Prepare 250 mL binding or elution buffers with final concentrations, 20 mM sodium phosphate, 500 mM NaCl and 10 to 500 mM imidazole:

Step	Action
1	Take 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).
2	Add X mL 2 M imidazole stock solution (see *Table 3, on page 12*). The volume of imidazole stock solution added depends on the chosen imidazole binding and elution concentrations.
3	Add distilled water to 200 mL and dissolve completely.
4	Adjust to pH 7.4 with HCl.
5	Add distilled water to 250 mL.
6	Filter through a 0.45 µm filter.

Table 3. Phosphate buffers (250 mL) with different imidazole concentrations.

Final imidazole concentration	2M imidazole stock solution volume (mL)
10	1.25

Final imidazole concentration	2M imidazole stock solution volume (mL)
20	2.50
30	3.75
40	5.00
50	6.25
60	7.50
70	8.75
80	10.00
90	11.25
100	12.50
300	37.50
500	62.50

7 Tips and hints

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

- Add detergents, reducing agents or other additives (Table 2) and mix gently for 30 min to aid solubilization of the tagged protein. Note that 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 (andunfolded) from inclusion bodies using common denaturants such as 4 to 6 M guanidine-HCl, 4 to 8 M urea or strong detergents. Mix gently for 30 min or more to aid solubilization.

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 to 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, on page 6*). 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% 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.

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.

8 Ordering information

Product	Pack size	Product Code
His SpinTrap	50 × 100 µL	28401353
His SpinTrap Kit[1]	1	28932171

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

Related products	Pack size	Product Code
His Buffer Kit	1	11003400
His GraviTrap™	10 × 1 mL	11003399
His GraviTrap Kit[1]	1	28401351
His MultiTrap™ FF	4 × prepacked 96-well plates	28400990
His MultiTrap HP	4 × prepacked 96-well plates	28400989
HisTrap™ FF, 1 mL	5 × 1 mL	17531901
HisTrap FF, 5 mL	5 × 5 mL	17525501
HisTrap FF crude, 1 mL	5 × 1 mL	11000458
HisTrap FF crude, 5 mL	5 × 5 mL	17528601
HisTrap HP, 1 mL	5 × 1 mL	17524701
HisTrap HP, 5 mL	1 × 5 mL	17524801
HisTrap HP, 5 mL	5 × 5 mL	17524802
Sepharose 6 Fast Flow	5 mL	17531806
Sepharose 6 Fast Flow	25 mL	17531801
Sepharose 6 Fast Flow	100 mL	17531802
Sepharose High Performance	25 mL	17526801
Sepharose High Performance	100 mL	17526802
Empty Disposable PD-10 columns	50	17043501
LabMate PD-10 Buffer Reservoir	10	18321603

[1] Includes 2 packs His GraviTrap and 1 pack His Buffer Kit

Literature	Product Code
Sepharose and IMAC Sepharose, Selection guide	28407092
Affinity Chromatography Columns and Media, Selection guide	18112186
Affinity Chromatography Handbook, Principle and Methods	18102229
Recombinant Protein Purification Handbook	18114275
Data File His SpinTrap and His SpinTrap Kit	28404659

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