GE Healthcare
Life Sciences

His Mag Sepharose™ Ni

His Mag Sepharose Ni products are magnetic beads designed for simple small-scale purification of histidine-tagged proteins. The magnetic beads are suitable for purification of a single sample or multiple samples in parallel, for example in screening experiments.

His Mag Sepharose Ni is available in the following pack sizes:

- His Mag Sepharose Ni, 5% medium slurry, 2 × 1 ml
- His Mag Sepharose Ni, 5% medium slurry, 5 × 1 ml
- His Mag Sepharose Ni, 5% medium slurry, 10 × 1 ml

Note: *1 ml medium slurry is sufficient for 5 reactions according to the recommended protocol. 1 ml of 5% (v/v) medium slurry contains 50 µl magnetic beads.*

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Please read these instructions carefully before use.

Intended use
His Mag Sepharose Ni is intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety
Contains nickel. For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

1 Product Description

General

His Mag Sepharose Ni products are magnetic beads designed for efficient, small-scale purification/screening of histidine-tagged proteins from different sources. Histidine-tagged proteins are captured using immobilized nickel ions followed by collection of the beads using a magnetic rack, such as MagRack 6 or MagRack Maxi (see Section 2). The magnetic beads are easily separated from the liquid phase during the different steps in the purification protocol. His Buffer Kit can be used to facilitate buffer preparation.

His Mag Sepharose Ni provides flexibility through the use of a wide range of sample and bead volumes. Purifications can be easily scaled up, and multiple samples can be processed in parallel.

Characteristics

His Mag Sepharose Ni characteristics

Matrix	Highly cross-linked spherical agarose (Sepharose), including magnetite
Particle size range	37 to 100 µm
Metal ion capacity	Approx. 21 µmol Ni^{2+}/ml medium
Binding capacity [1]	Approx. 50 mg $(histidine)_6$-tagged protein/ml sedimented medium (~500 µg/purification run using the standard protocol)
Working temperature	4°C to 30°C
Storage solution	20% ethanol, 5% medium slurry
Storage temperature	Room temperature

[1] The capacity was determined using 5 mM imidazole in sample and equilibration buffer. Note that binding capacity is sample-dependent.

Compatibility

His Mag Sepharose Ni compatibility

His Mag Sepharose Ni is compatible with all commonly used aqueous buffers, reducing agents, denaturants such as 6 M guanidine hydrochloride, 8 M urea, and a range of other additives.

Reducing agents	5 mM DTE 5 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione
Denaturing agents	8 M urea [1] 6 M guanidine hydrochloride [1]
Detergents	2% Triton™ X-100 (nonionic) 2% Tween 20 (nonionic) 2% NP-40 (nonionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA [2] 60 mM citrate [2]
Buffer substances	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 sodium acetate, pH 4.0 [1]

[1] Tested for 1 week at 40°C.

[2] The strong chelator EDTA has been used successfully in some cases at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not the buffer). Any metal-ion stripping may be counteracted by adding a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with the applied sample volume.

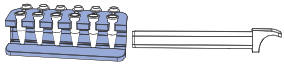
2 Handling

Note: *His Mag Sepharose Ni is intended for single use only.*

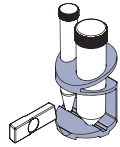
General magnetic separation step

When performing magnetic separation, it is recommended to use MagRack 6 for test tubes up to 1.5 ml and MagRack Maxi for test tubes up to 50 ml.

1 *Remove* the magnet before *adding* liquid

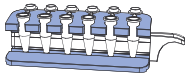


MagRack 6

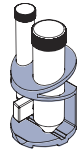


MagRack Maxi

2 *Insert* the magnet before *removing* liquid



MagRack 6



MagRack Maxi

When using volumes above 50 ml, the beads can be spun down using a swing-out centrifuge.

Dispensing the bead slurry

- Prior to dispensing the bead slurry, make sure it is homogeneous by vortexing the vial thoroughly.
- When the beads are resuspended, *immediately* pipette the required amount of bead slurry into the desired tube.
- Due to the fast sedimentation of the beads, it is important to repeat the resuspension between each pipetting.

Handling of liquid

- Before application of liquid, remove the magnet from the magnetic rack.
- After addition of liquid, resuspend the beads by vortexing or manual inversion of the tube. When processing multiple samples, manual inversion of the magnetic rack is recommended.
- Use the magnetic rack with the magnet in place for each liquid removal step. Pipette or pour off the liquid.
- If needed, a pipette can be used to remove liquid from the lid of the test tube.

Incubation

- During incubation, make sure the magnetic beads are well resuspended and kept in solution by end-over-end mixing or by using a benchtop shaker.
- When purifying samples of large volumes, an increase of the incubation time may be necessary.

3 Operation

Recommended buffers

Note: *Use high-purity water and chemicals for buffer preparation.*

Equilibration buffer
20 mM sodium phosphate, 500 mM NaCl, 5 to 60 mM imidazole, pH 7.4

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

Note: *See Section Purification protocols for recommended imidazole concentrations in equilibration buffer.*

Note: *Suitable buffers can be easily prepared using His Buffer Kit (see Section Ordering information).*

Sample preparation

- Adjust the sample to the composition and pH of the equilibration buffer. This can be done by adding buffer, NaCl, and imidazole from stock solutions; by diluting with equilibration buffer or by buffer exchange using PD MiniTrap™ G-25 or, depending on sample volume, another disposable desalting column of suitable size, see www.gelifesciences.com/sampleprep.
- Clarification of sample may be needed before applying it to the beads.

4 Purification protocols

General

Purification of histidine-tagged proteins by immobilized metal affinity chromatography (IMAC) is a balance between yield and purity. Low concentration of imidazole in the sample and equilibration buffer promotes high yield while higher imidazole concentrations increases purity. Note also that optimal imidazole concentration is sample-dependent. To simplify the purification procedure, two purification protocols are included in this instruction, one with focus on high capacity and the other one with focus on high purity.

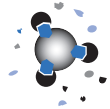
Purification protocol for high capacity

This protocol is suitable when the first priority is obtaining large amounts of histidine-tagged protein. High capacity is obtained using a low imidazole concentration during equilibration and wash.

When using MagRack 6 or MagRack Maxi for magnetic separation, please refer to Section *Handling* for correct handling of the magnetic rack.

Step	Action
A	**Magnetic bead preparation**
1	Mix the bead slurry thoroughly by vortexing. Dispense 200 µl homogeneous slurry into a 1.5 ml test tube.
2	Place the test tube in the magnetic rack (see Section *Handling*).
3	Remove the storage solution.
B	**Equilibration**
1	Add 500 µl equilibration buffer containing 5 mM imidazole.
2	Resuspend the beads.
3	Remove the liquid.

Step	Action
C	**Sample application**
1	Immediately after equilibration, add 1000 µl sample containing 5 mM imidazole. If the sample volume is less than 1000 µl, dilute to 1000 µl with equilibration buffer.
2	Resuspend the beads and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.
3	Remove the liquid.
D	**Washing**
1	Add 500 µl equilibration buffer containing 5 mM imidazole.
2	Resuspend the beads.
3	Remove the liquid.
4	Wash three times in total.
E	**Elution**
1	Add 100 µl elution buffer.
2	Resuspend the beads.
3	Collect the elution fraction. The collected elution fraction contains the main part of the purified protein. If desired, repeat the elution.



Purification protocol for high purity

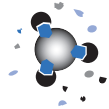
This protocol is suitable when the first priority is obtaining highly pure histidine-tagged protein. High purity is obtained using higher imidazole concentrations (20 to 60 mM) during equilibration and wash.

Note: *The optimal imidazole concentration is protein dependent. 20 mM may be optimal for weak-binding histidine-tagged proteins while 60 mM may be optimal for strong-binding histidine-tagged proteins.*

When using MagRack 6 or MagRack Maxi for magnetic separation, please refer to Section *Handling* for correct handling of the magnetic rack.

Step	Action
A	**Magnetic bead preparation**
1	Mix the bead slurry thoroughly by vortexing. Dispense 200 µl homogeneous slurry into a 1.5 ml test tube.
2	Place the test tube in the magnetic rack (see Section *Handling*).
3	Remove the storage solution.
B	**Equilibration**
1	Add 500 µl equilibration buffer containing 20 to 60 mM imidazole.
2	Resuspend the beads.
3	Remove the liquid.

Step	Action
C	**Sample application**
1	Immediately after equilibration, add 1000 µl sample containing 20 to 60 mM imidazole. If the sample volume is less than 1000 µl, dilute to 1000 µl with equilibration buffer.
2	Resuspend the beads and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.
3	Remove the liquid.
D	**Washing**
1	Add 500 µl equilibration buffer containing 20 to 60 mM imidazole.
2	Resuspend the beads.
3	Remove the liquid.
4	Wash three times in total.
E	**Elution**
1	Add 100 µl elution buffer.
2	Resuspend the beads.
3	Collect the elution fraction. The collected elution fraction contains the main part of the purified protein. If desired, repeat the elution.



5 Optimization

The protocols recommended in this instruction are suitable for purification of most histidine-tagged proteins. Some conditions may require optimization.

Examples of conditions which may require optimization are:

- sample volume
- amount of beads
- sample incubation time
- imidazole concentration during wash
- number of washes
- buffer composition, pH etc.

6 Ordering information

Products

Product	Quantity	Code No.
His Mag Sepharose Ni	2 × 1 ml 5% medium slurry	28-9673-88
	5 × 1 ml 5% medium slurry	28-9673-90
	10 × 1 ml 5% medium slurry	28-9799-17

Related products

Products	Quantity	Code No.
MagRack 6	1	28-9489-64
MagRack Maxi	1	28-9864-41
His Buffer Kit	1	11-0034-00
His Mag Sepharose excel	2 × 1 ml 10% medium slurry	17-3712-20
	5 × 1 ml 10% medium slurry	17-3712-21
	10 × 1 ml 10% medium slurry	17-3712-22
PD MiniTrap G-25	50	28-9180-07
PD-10 Desalting Column	30	17-0851-01

Related literature

Related literature	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Data file, His Mag Sepharose Ni	28-9797-23

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/sampleprep

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent numbers 5,284,933 and 5,310,663, and equivalent patents and patent applications in other countries (assignee: Hoffman La Roche, Inc).

These products are sold under a license from Sigma-Aldrich under patent number EP 1277616 (Metal chelating compositions) and equivalent patents and patent applications in other countries.

Triton is a trademark of Union Carbide Chemicals and Plastic Company Inc.

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GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

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His Mag Sepharose Ni
Purification protocol for high capacity

Step	Action
A	**Magnetic bead preparation**
1	Mix the bead slurry thoroughly by vortexing. Dispense 200 µl homogeneous slurry into a 1.5 ml test tube.
2	Place the test tube in the magnetic rack (see Section *Handling*).
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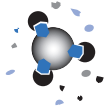
3	Remove the liquid.



Step	Action	
D	**Washing**	
	1 Add 500 µl equilibration buffer containing 5 mM imidazole.	
	2 Resuspend the beads.	
	3 Remove the liquid.	
	4 Wash three times in total.	
E	**Elution**	
	1 Add 100 µl elution buffer.	
	2 Resuspend the beads.	
	3 Collect the elution fraction. The collected elution fraction contains the main part of the purified protein. If desired, repeat the elution.	

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Purification protocol for high purity

Step	Action

A Magnetic bead preparation

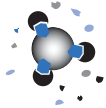
1. Mix the bead slurry thoroughly by vortexing. Dispense 200 µl homogeneous slurry into a 1.5 ml test tube.
2. Place the test tube in the magnetic rack (see Section *Handling*).
3. Remove the storage solution.

B Equilibration

1. Add 500 µl equilibration buffer containing 20 to 60 mM imidazole.
2. Resuspend the beads.
3. Remove the liquid.

C Sample application

1. Immediately after equilibration, add 1000 µl sample containing 20 to 60 mM imidazole. If the sample volume is less than 1000 µl, dilute to 1000 µl with equilibration buffer.
2. Resuspend the beads and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.
3. Remove the liquid.



Step	Action	
D	**Washing**	
	1 Add 500 µl equilibration buffer containing 20 to 60 mM imidazole.	
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