GE Healthcare
Life Sciences

His Mag Sepharose™ excel

His Mag Sepharose excel is a magnetic immobilized metal ion affinity chromatography (IMAC) medium (resin) charged with nickel ions.

The magnetic beads are designed for simple small-scale capture and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. With nickel ions exceptionally strongly bound to a chelating ligand, His Mag Sepharose excel is optimal for samples usually causing stripping of metal ions from conventional IMAC media.

The beads may be used for purification of a single sample or multiple samples in parallel, for example in screening experiments.

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

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

Note: *1 ml medium slurry is sufficient for 5 reactions according to the recommended protocol. 1 ml of 10% (v/v) medium slurry contains 100 µl magnetic beads.*

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Please read these instructions carefully before use.

Intended use
His Mag Sepharose excel 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

Background

Traditionally, IMAC purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants is often problematic due to incompatibility with the cell culture media, leading to stripping of the immobilized metal ions during sample application. This results in low or no binding of the target protein.

The purification is further complicated by the fact that the target protein concentration often is low. This requires large sample volumes to be used, which in turn may lead to increased metal ion stripping.

To overcome these problems, considerable sample pretreatment has been required, such as buffer exchange by ultrafiltration in combination with concentration procedures. Such pretreatment is time consuming and potentially harmful to sensitive proteins.

With His Mag Sepharose excel, pretreatment procedures can be reduced to a minimum.

General description

His Mag Sepharose excel is a magnetic medium for IMAC protein purification. The beads are precharged with nickel ions exceptionally strongly bound to a chelating ligand. The nickel ions remain bound to the chelating ligand even after 24 hours incubation in 10 mM EDTA.

Samples usually causing stripping of metal ions can be safely applied to the medium without extensive and time-consuming pretreatment procedures. Examples of samples that often cause stripping problems are cell culture supernatants containing secreted histidine-tagged proteins from eukaryotic cells, such as insect cells or CHO cells. His Mag Sepharose excel is also suitable for purification of histidine-tagged proteins from other samples causing extensive metal ion stripping from conventional IMAC media.

For samples without extensive stripping effects, it is recommended to use His Mag Sepharose Ni, which normally show higher affinity for histidine-tagged proteins.

Characteristics

Mag Sepharose products provide flexible purification allowing a wide range of sample volumes, the ability for purification of multiple samples in parallel and easy scale-up by varying the bead quantity. His Mag Sepharose excel can be used together with different test tubes and magnetic racks, for example MagRack 6 for test tubes up to 1.5 ml or MagRack Maxi for test tubes up to 50 ml (see Section *Handling*). His Buffer Kit can be used to facilitate buffer preparation.

The key characteristics of His Mag Sepharose excel are listed in the following table.

His Mag Sepharose excel characteristics

Matrix	Highly cross-linked spherical agarose (Sepharose), including magnetite
Particle size range	37 to 100 µm
Metal ion capacity	30 to 42 µmol Ni^{2+}/ml medium
Binding capacity [1]	At least 10 mg $(histidine)_6$-tagged protein/ml sedimented medium (~ 200 µg/purification run using the standard protocol)
Working temperature	4°C to 30°C
Compatibility during use	Stable in buffers commonly used in IMAC
Chemical stability [2]	
- 0.01 M HCl, and 0.01 M NaOH	Tested for one week
- 10 mM EDTA, 1 M NaOH, 5 mM DTT, 5 mM TCEP, 20 mM β-mercaptoethanol, and 6 M guanidine-HCl	Tested for 24 hours
- 500 mM imidazole, and 100 mM EDTA	Tested for 2 hours
- 30% 2-propanol	Tested for 20 minutes
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	4°C to 30°C

1 Binding capacity is sample-dependent.

2 Chemical stability of the product was tested by incubating His Mag Sepharose excel or Ni Sepharose excel (which has the same ligand) in the listed solutions at room temperature and thereafter measuring either the nickel leakage or the protein binding capacity.

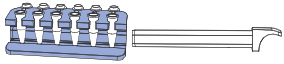
2 Handling

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

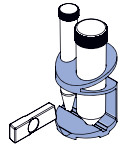
General magnetic separation step

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

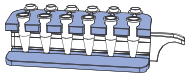


MagRack 6

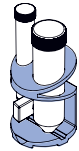


MagRack Maxi

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 Buffer preparation

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

Recommended buffers

Equilibration buffer

20 mM sodium phosphate, 0.5 M NaCl, pH 7.4

Wash buffer

20 mM sodium phosphate, 0.5 M NaCl, 0 to 30 mM imidazole, pH 7.4

Elution buffer

20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

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

Note: *It is not recommended to include imidazole in sample and equilibration buffer.*

Buffer optimization

Imidazole concentration in wash buffers

To minimize host cell proteins in the eluate, it is recommended to include imidazole at low concentrations in the wash buffer. However, for some target proteins, even a small increase of the imidazole concentration in the wash buffer may lead to partial elution.

The imidazole concentration must therefore be optimized to ensure the best balance of high purity (low binding of host cell proteins) and high yield (strong binding of histidine-tagged target protein). For high purity, a higher imidazole concentration is required in the wash buffer, and insect cell culture supernatants generally require higher imidazole concentration than CHO cell culture supernatants.

The concentration of imidazole that will give optimal purification results is sample-dependent, and is usually lower for His Mag Sepharose excel than for other Ni Sepharose media used for IMAC purification. Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and-error effort, but 10 to 20 mM in the wash buffer is a good starting range for many proteins.

If the wash buffer contains imidazole, elution should be performed immediately after wash, since leaving the sample in wash buffer may cause loss of the target protein.

4 Sample preparation

His Mag Sepharose excel allows application of unclarified cell culture supernatants. However, before sample application, whole cells must be removed by, for example, centrifugation.

For optimal binding, it is **not** recommended to include imidazole in sample and equilibration buffer. It is recommended to perform binding at neutral pH. However, successful purification has routinely been observed with binding performed at a pH as low as 6.0. Due to the precipitation risk, avoid using strong bases or acids for pH-adjustments.

5 Purification protocol

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.
2	Resuspend the beads.
3	Remove the liquid.

Step	Action
C	**Sample application**
	1 Immediately after equilibration, transfer the beads into a larger test tube containing 10 ml sample.
	2 Resuspend the beads and incubate for 4 hours with slow end-over-end mixing.
	3 Remove the liquid.
	Note: *For higher recovery when purifying weakly binding proteins, it is recommended to extend the incubation time to overnight.*
D	**Washing**
	1 Add 500 µl wash buffer and retransfer the beads to a 1.5 ml test tube.
	2 Resuspend the beads.
	3 Remove the liquid.
	4 Wash three times in total.
	Note: *If the wash buffer contains imidazole, do not leave the beads in wash buffer for longer than 1 minute since this may lead to loss of the target protein.*
E	**Elution**
	1 Add 200 µl elution buffer.
	2 Resuspend the beads and incubate for 1 minute with occasional mixing on a vortex mixer.
	3 Collect the elution fraction. The collected elution fraction contains the main part of the purified protein. For optimal recovery, repeat the elution.

6 Optimization

The protocol recommended in this instruction should be regarded as a starting point for purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. 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.

For more information, see Section *Buffer preparation*.

7 Ordering information

Products

Product	Quantity	Code No.
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

Related products

Products	Quantity	Code No.
HisTrap™ excel	5 × 1 ml	17-3712-05
	5 × 5 ml	17-3712-06
Ni Sepharose excel	100 ml	17-3712-02
	500 ml	17-3712-03
MagRack 6	1	28-9489-64
MagRack Maxi	1	28-9864-41
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
PD MiniTrap™ G-25	50 columns	28-9180-07
His Buffer Kit	1	11-0034-00

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 excel, Ni Sepharose excel, HisTrap excel	29-0168-49

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

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).

First published Apr. 2012

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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29-0163-14 AC 04/2012

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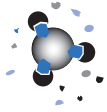

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1	Add 500 µl wash buffer and retransfer the beads to a 1.5 ml test tube.
2	Resuspend the beads
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4	Wash three times in total.

Note:
If the wash buffer contains imidazole, do not leave the beads in wash buffer for longer than 1 minute since this may lead to loss of the target protein.

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