His Mag Sepharose<sup>TM</sup> Ni

His Mag Sepharose Ni is available in the following pack sizes (instructions for use included in all 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.

Purpose

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.

Intended use

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

CAUTION: The product contains nickel, Ni<sup>2+</sup>, which is potentially allergenic. Always use normal protection devices like gloves and safety glasses when handling His Mag Sepharose Ni.
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1 Principle

His Mag Sepharose Ni is an affinity chromatography medium with high affinity for histidine-tagged proteins from various sources. The medium consists of magnetic beads based on Sepharose coupled with IMAC ligand and immobilized with nickel ions. Purification of histidine-tagged proteins on IMAC media is a balance between capacity and purity, modulated by the concentration of imidazole in the sample and in the binding/wash buffer. 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.

His Mag Sepharose Ni provides flexible purification allowing a wide range of sample volumes and easy scale-up by varying the bead quantity and is suitable for purification of multiple samples in parallel.

Mag Sepharose products can be used together with Eppendorf microcentrifuge tubes and a magnetic rack, for example MagRack 6 (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.
2 Advice on handling

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

General magnetic separation step
It is recommended to use 1.5 ml Eppendorf tubes and MagRack 6 in the included protocol (see Section 4 and Section 5).

1 Remove the magnet before adding liquid.

2 Insert the magnet before removing liquid.

When using volumes above 1.5 ml, e.g. 50 ml, a magnetic pickpen can be used for collecting the magnetic beads. Another alternative is to spin down the beads by using a swing-out centrifuge.

Dispensing the medium slurry

- Prior to dispensing the medium slurry, make sure it is homogeneous by vortexing the vial thoroughly.
- When the medium slurry is resuspended, immediately pipette the required amount of medium slurry into the desired tube.
- Repeat the resuspension step between each pipetting from the medium slurry vial.
Handling of liquid

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

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.
- Incubation generally takes place at room temperature. However, incubation can take place at +4°C if this is the recommended condition for the specific sample.
- 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.

**Table 1.** Recommended buffers.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding/buffer</td>
<td>20 mM sodium phosphate, 500 mM NaCl, 5-60 mM imidazole, pH 7.4</td>
</tr>
<tr>
<td>Elution buffer</td>
<td>20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4</td>
</tr>
</tbody>
</table>

1. See protocols for recommended imidazole concentrations in binding/wash buffer.

- Suitable buffers can also be easily prepared using His Buffer Kit (see Section 9).
- His Mag Sepharose Ni permits use of a variety of buffers and solutions (see Table 3).

Sample pretreatment

- Adjust the sample to the composition and pH of the binding buffer. This can be done by adding buffer, NaCl and imidazole from stock solutions, by diluting with binding buffer or by buffer exchange using PD MiniTrap™ G-25 or HiTrap™ Desalting columns.
- Clarification of sample may be needed before applying it to the beads.
4 Purification protocol for high capacity

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

1 Magnetic bead preparation
   A Mix the medium slurry thoroughly by vortexing. Dispense 200 µl of suspended magnetic beads into an Eppendorf tube.
   B Place the Eppendorf tube in the magnetic rack (see Section 2).
   C Remove the storage solution.

2 Equilibration
   A Add 500 µl binding buffer containing 5 mM imidazole.
   B Resuspend the medium.
   C Remove the liquid.

3 Sample application
   A Immediately after equilibration, add 1000 µl of sample containing 5 mM imidazole. If the sample volume is less than 1000 µl, dilute to 1000 µl with binding buffer.
   B Resuspend the medium and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.
   C Remove the liquid.

4 Washing (perform this step 3 times totally)
   A Add 500 µl binding buffer containing 5 mM imidazole.
   B Resuspend the medium.
   C Remove the liquid.

5 Elution
   A Add 100 µl of elution buffer.
   B Resuspend the medium.
   C Remove and collect the elution fraction. The collected elution fraction contains the main part of the purified protein. If desired, repeat the elution.
5 Purification protocol for high purity

This protocol is suitable when high purity of histidine-tagged protein is first priority. High purity is obtained using higher imidazole concentrations (20-60 mM) during binding 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.

1 Magnetic bead preparation
   A Mix the medium slurry thoroughly by vortexing. Dispense 200 µl of suspended magnetic beads into an Eppendorf tube.
   B Place the Eppendorf tube in the magnetic rack (see Section 2).
   C Remove the storage solution.

2 Equilibration
   A Add 500 µl binding buffer with 20-60 mM imidazole.
   B Resuspend the medium.
   C Remove the liquid.

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

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   A Add 500 µl binding buffer with 20-60 mM imidazole.
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   C Remove the liquid.

5 Elution
   A Add 100 µl of elution buffer.
   B Resuspend the medium.
   C Remove and collect the elution fraction. The collected elution fraction contains the main part of the purified protein. If desired, repeat the elution.
6 Optimization of parameters

The protocols recommended in this instruction (see Section 4 and Section 5) are suitable for purification of most histidine-tagged proteins. However, some parameters for purification may require optimization to obtain the best result.

Examples of parameters which may require optimization are:

- Amount of beads
- Incubation times
- Number of washes
- Imidazole concentration in sample and binding buffer
- Buffer composition, pH etc.

7 Characteristics

Table 2. His Mag Sepharose Ni.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix</td>
<td>Highly crosslinked spherical agarose (Sephase) including magnetite</td>
</tr>
<tr>
<td>Metal ion capacity</td>
<td>~21µmol Ni²⁺/ml medium</td>
</tr>
<tr>
<td>Binding capacity¹</td>
<td>Approx. 50 mg histidine-tagged protein/ml sedimented medium (~500 µg/purification run)</td>
</tr>
<tr>
<td>Particle size</td>
<td>37 to 100 µm</td>
</tr>
<tr>
<td>Working temperature</td>
<td>Room temperature and +4°C</td>
</tr>
<tr>
<td>Storage solution</td>
<td>20% ethanol, 5% medium slurry</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

¹ The capacity was determined using 5 mM imidazole in sample and binding buffer. Note that binding capacity is sample dependent.
## 8 Compatibility

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

**Table 3.** His Mag Sepharose Ni is compatible with the following compounds, at least at the concentrations given.

<table>
<thead>
<tr>
<th>Category</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing agents</td>
<td>5 mM DTE&lt;br&gt;5 mM DTT&lt;br&gt;5 mM TCEP&lt;br&gt;10 mM reduced glutathione</td>
</tr>
<tr>
<td>Denaturing agents</td>
<td>8 M urea&lt;br&gt;6 M guanidine hydrochloride</td>
</tr>
<tr>
<td>Detergents</td>
<td>2% Triton X-100 (nonionic)&lt;br&gt;2% Tween 20 (nonionic)&lt;br&gt;2% NP-40 (nonionic)&lt;br&gt;2% cholate (anionic)&lt;br&gt;1% CHAPS (zwitterionic)</td>
</tr>
<tr>
<td>Other additives</td>
<td>500 mM imidazole&lt;br&gt;20% ethanol&lt;br&gt;50% glycerol&lt;br&gt;100 mM Na₂SO₄&lt;br&gt;1.5 M NaCl&lt;br&gt;1 mM EDTA&lt;br&gt;60 mM citrate</td>
</tr>
<tr>
<td>Buffer substances</td>
<td>50 mM sodium phosphate, pH 7.4&lt;br&gt;100 mM Tris-HCl, pH 7.4&lt;br&gt;100 mM Tris-acetate, pH 7.4&lt;br&gt;100 mM HEPES, pH 7.4&lt;br&gt;100 mM MOPS, pH 7.4&lt;br&gt;sodium acetate, pH 4.0</td>
</tr>
</tbody>
</table>

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 MgCl₂ before centrifugation/filtration of the sample. Note that stripping effects may vary with the applied sample volume.
## 9 Ordering information

<table>
<thead>
<tr>
<th>Products</th>
<th>Quantity</th>
<th>Code No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>His Mag Sepharose Ni</td>
<td>2 × 1 ml 5% medium slurry</td>
<td>28-9673-88</td>
</tr>
<tr>
<td>His Mag Sepharose Ni</td>
<td>5 × 1 ml 5% medium slurry</td>
<td>28-9673-90</td>
</tr>
<tr>
<td>His Mag Sepharose Ni</td>
<td>10 × 1 ml 5% medium slurry</td>
<td>28-9799-17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Related products</th>
<th>Quantity</th>
<th>Code No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagRack 6</td>
<td>1</td>
<td>28-9489-04</td>
</tr>
<tr>
<td>His Buffer Kit</td>
<td>1</td>
<td>11-0034-00</td>
</tr>
<tr>
<td>HiTrap Desalting</td>
<td>5 × 5 ml</td>
<td>17-1408-01</td>
</tr>
<tr>
<td>PD MiniTrap G-25</td>
<td>50 columns</td>
<td>28-9180-07</td>
</tr>
<tr>
<td>Protein A Mag Sepharose</td>
<td>1 × 500 µl 20% medium slurry</td>
<td>28-9440-06</td>
</tr>
<tr>
<td>Protein A Mag Sepharose</td>
<td>4 × 500 µl 20% medium slurry</td>
<td>28-9513-78</td>
</tr>
<tr>
<td>Protein A Mag Sepharose Xtra 2</td>
<td>2 × 1 ml 10% medium slurry</td>
<td>28-9670-56</td>
</tr>
<tr>
<td>Protein A Mag Sepharose Xtra 5</td>
<td>1 × 1 ml 10% medium slurry</td>
<td>28-9670-62</td>
</tr>
<tr>
<td>Protein G Mag Sepharose</td>
<td>1 × 500 µl 20% medium slurry</td>
<td>28-9440-08</td>
</tr>
<tr>
<td>Protein G Mag Sepharose</td>
<td>4 × 500 µl 20% medium slurry</td>
<td>28-9513-79</td>
</tr>
<tr>
<td>Protein G Mag Sepharose Xtra 2</td>
<td>1 × 1 ml 10% medium slurry</td>
<td>28-9670-66</td>
</tr>
<tr>
<td>Protein G Mag Sepharose Xtra 5</td>
<td>1 × 1 ml 10% medium slurry</td>
<td>28-9670-70</td>
</tr>
<tr>
<td>NHS Mag Sepharose</td>
<td>1 × 500 µl 20% medium slurry</td>
<td>28-9440-09</td>
</tr>
<tr>
<td>NHS Mag Sepharose</td>
<td>4 × 500 µl 20% medium slurry</td>
<td>28-9513-80</td>
</tr>
<tr>
<td>TiO2 Mag Sepharose</td>
<td>1 × 500 µl 20% medium slurry</td>
<td>28-9440-10</td>
</tr>
<tr>
<td>TiO2 Mag Sepharose</td>
<td>4 × 500 µl 20% medium slurry</td>
<td>28-9513-77</td>
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