His Mag Sepharose™ Ni

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 device. You can use magnetic beads to simplify sample handling during small-scale purification.

His Mag Sepharose Ni delivers:

- High-capacity small-scale purification of histidine-tagged proteins from different sources
- High purity and yield
- Easy parallel screening of histidine-tagged proteins with high repeatability
- Scalability—Simple capture of histidine-tagged proteins from small or large sample volumes (low microliter to milliliter scale)

The beads are available in three pack sizes: 2 × 1 ml 5% medium slurry, 5 × 1 ml 5% medium slurry, and 10 × 1 ml 5% medium slurry—a 1 ml 5% medium slurry is the same as 50 µl sedimented medium. A 1 ml medium slurry is sufficient for 5 purification runs according to the recommended protocol. Together with MagRack 6, a separation tool for handling the beads in microcentrifuge tubes, up to six samples can be processed in parallel. You can easily screen a larger number of samples in parallel with high throughput on a robotic device.

Simplified handling

The magnetic bead format has excellent properties for small-scale experiments. The high density of the beads allows rapid capture by magnetic devices while the visibility of the beads ensures reliable collection of the bound histidine-tagged proteins in the purification procedure. The characteristics of the media are summarized in Table 1. All the products are provided with protocols optimized for purification of histidine-tagged proteins.

Fig 1. His Mag Sepharose Ni is designed for the purification/screeening of histidine-tagged proteins from cell lysates and cell supernatants.

Table 1. Characteristics of His Mag Sepharose Ni

Matrix	Highly crosslinked spherical agarose (Sepharose) including magnetite
Metal ion capacity	~ 21 µmol Ni^{2+}/ml medium
Binding capacity[1]	approx. 50 mg histidine-tagged protein/ml medium (approx 500 µg/purification run)
Particle size	37 to 100 µm
Working temperature	Room temperature and + 4° C
Storage solution	20% ethanol, 5% medium slurry
Storage temperature	Room temperature

[1]Binding capacity is protein dependent

MagRack 6 is a magnetic rack that allows you to prepare up to six samples captured in 1.5 ml microcentrifuge tubes. When the tubes are placed in the rack, the magnetic beads are attracted to the magnet within a few seconds. This allows you to easily remove the supernatant while the magnetic beads are retained in the tube.

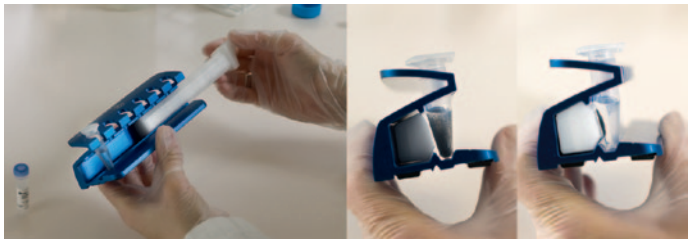

Fig 2. The high density of the beads allows rapid capture by MagRack 6 magnetic device.

His Buffer Kit can be used to eliminate time-consuming buffer preparations and this leads to fast and reproducible purification.

Optimal purification of histidine-tagged proteins

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 binding buffer promotes high yield while higher imidazole concentrations increases purity. The establishment of an optimal imidazole concentration is protein dependent.

At GE Healthcare Laboratories, we examined the effect of different imidazole concentrations on the purification of a histidine-tagged protein X in *Pichia Pastoris* lysate using His Mag Sepharose Ni and the corresponding products from Qiagen™ and Millipore™ (Table 2).

We purified the target protein according to the manufacturer's instructions in the presence of 5, 20, 40, 60, and 80 mM imidazole in both the sample and binding/wash buffers. The purified samples were analyzed by SDS-PAGE analysis followed by purity determination (Fig 3). The results show that the lower the imidazole concentrations, the higher the amount of protein produced. On the other hand, increased imidazole concentrations led to a higher purity (Fig 3). In this comparative performance evaluation, His Mag Sepharose Ni produced higher yield and purity than the corresponding products from Qiagen and Millipore.

Table 2. Experimental conditions for the benchmark analysis experiment

Media	His Mag Sepharose Ni	Ni-NTA Magnetic Agarose Beads	PureProteome™ Nickel Magnetic Beads
Supplier	GE Healthcare (GEHC)	Qiagen	Millipore
Sample volume	1 ml	1 ml	1 ml
Load	200 µg target protein	200 µg target protein	200 µg target protein
Binding/wash buffer	20 mM sodium phosphate, 0.5 M NaCl and 5 to 80 mM imidazole pH 7.4	10 mM sodium phosphate, 0.3 M NaCl and 5 to 80 mM imidazole, pH 8.0	10 mM sodium phosphate, 0.3 M NaCl and 5 to 80 mM imidazole, pH 8.0
Elution buffer	20 mM sodium phosphate, 0.5 M NaCl, and 500 mM imidazole, pH 7.4	20 mM sodium phosphate, 0.3 M NaCl, and 500 mM imidazole, pH 8.0	10 mM sodium phosphate, 0.3 M NaCl, and 500 mM imidazole, pH 8.0

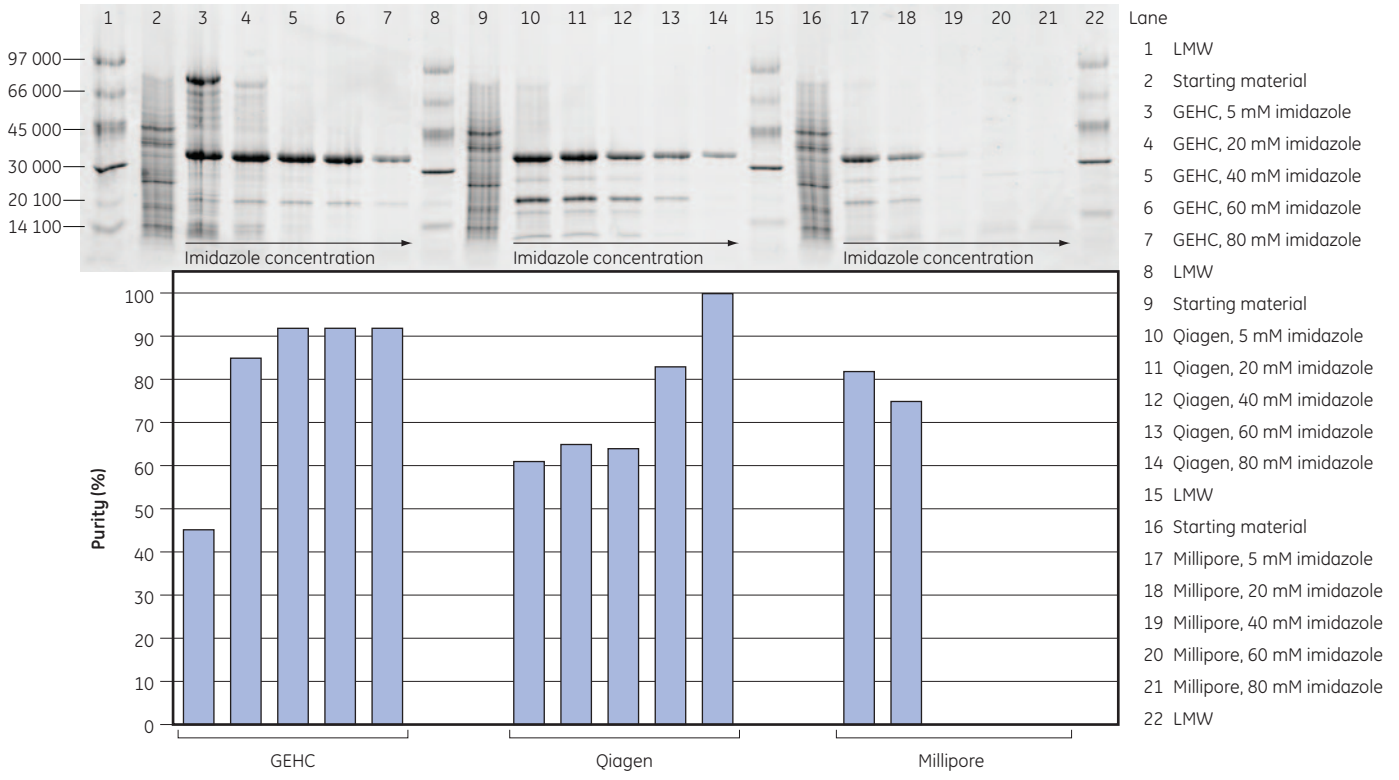

Fig 3. Purification of a histidine-tagged protein in *Pichia Pastoris* using different imidazole concentrations (5, 20, 40, 60, and 80 mM) in both the sample and binding/wash buffers. The SDS-PAGE at the top panel was stained with Deep Purple™ Total Protein Stain under reducing conditions and protein purity was measured with ImageQuant™ TL software.

Repeatable purification of a histidine-tagged protein

To show the efficiency and repeatability of His Mag Sepharose Ni, six replicate purification runs were performed (Table 3). The load was half of the total binding capacity for the media. The average protein yield was 230 µg (Fig 4) and the purity analyzed by SDS-PAGE was > 90% (Fig 5). High repeatability was demonstrated for both yield and purity using His Mag Sepharose Ni with a relative standard deviation (RSD) of < 2%.

Table 3. Experimental conditions for His Mag Sepharose Ni

Sample	GFP-$(His)_6$ in *E. coli* lysate
Sample volume	1 ml
Binding/wash buffer	20 mM sodium phosphate, 500 mM NaCl, 40 mM imidazole, pH 7.4
Elution buffer	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

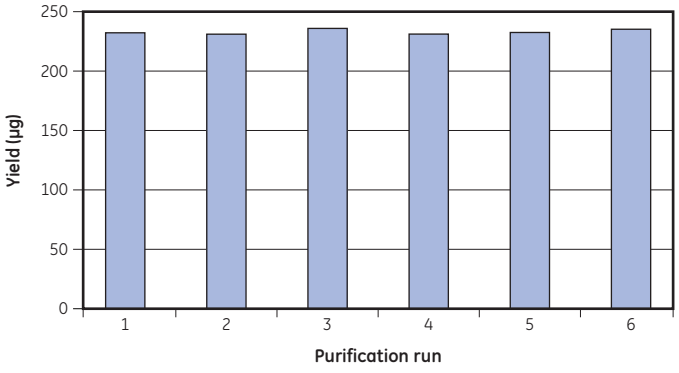

Fig 4. The average amount of histidine-tagged protein eluted for six replicate runs was 230 µg (RSD < 2%).

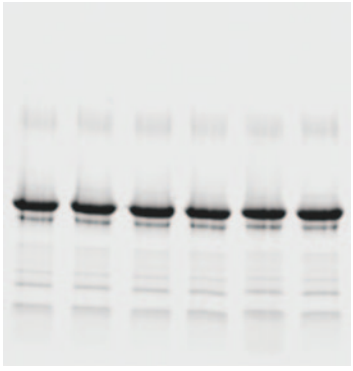

Fig 5. SDS-PAGE stained with Deep Purple Total Protein Stain. The six replicate runs produced purity levels of over 90% (RSD < 2%).

Purification of a cell wall-associated protein in *Lactococcus lactis*

A histidine-tagged cell wall-associated protein from *Staphylococcus aureus* was expressed in *Lactococcus lactis* and purified using His Mag Sepharose Ni. The primary goal was to obtain high purity, therefore 50 mM imidazole was included in the sample and binding buffer (Table 4). The SDS-PAGE shows a minor leakage of target protein during sample application and wash, as a result of the relatively high imidazole concentration. The purity of the eluted target protein was above 95% and the yield was approximately 600 µg purified protein (Fig 6).

Table 4. Experimental conditions for His Mag Sepharose Ni

Sample	Histidine-tagged protein Y in *L. lactis*
Sample volume	1 ml
Binding/wash buffer	20 mM sodium phosphate, 0.5 M NaCl, and 50 mM imidazole, pH 7.4
Elution buffer	20 mM sodium phosphate, 0.5 M NaCl, and 500 mM imidazole, pH 7.4

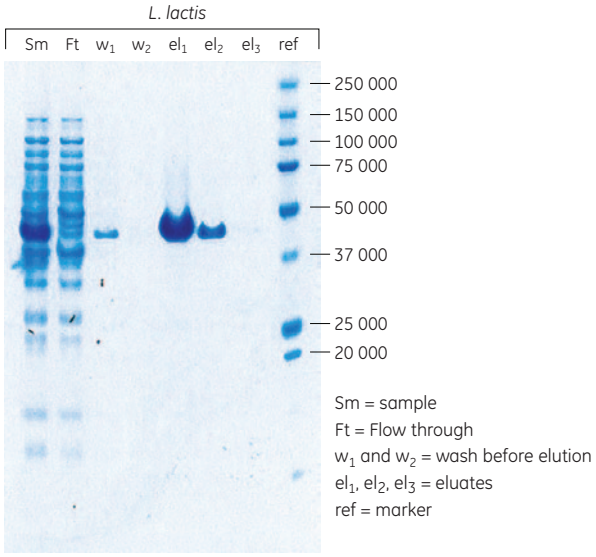

Fig 6. Purification of a cell wall protein in *L. lactis* resulted in high purity (> 95%) according to SDS gel stained with Coomassie.

Acknowledgement: The results for histidine-tagged protein purification in *L. Lactis* was kindly provided by Prof. Jan-Maarten van Dijl and Dennis Koedijk, University Medical Centre of Groningen (UMCG), Groningen, The Netherlands.

Flexible scale-up

One of the key advantages of magnetic bead purification is the ability to use different volumes of sample and medium slurry. Purification was scaled up tenfold from 200 µl to 2000 µl His Mag Sepharose Ni. The sample volume was 1 ml and 10 ml, respectively (Table 5). The load was 80% of the total binding capacity for the media. The yield of the target protein was 0.42 mg using 200 µl medium slurry and 4.3 mg using 2000 µl medium slurry. Purity as analyzed by SDS-PAGE was equally high for both purifications (Fig 7).

Table 5. Experimental conditions for His Mag Sepharose Ni

Sample	GFP-$(His)_6$ in *E. coli* lysate
Sample volume	1 ml or 10 ml
Binding/wash 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

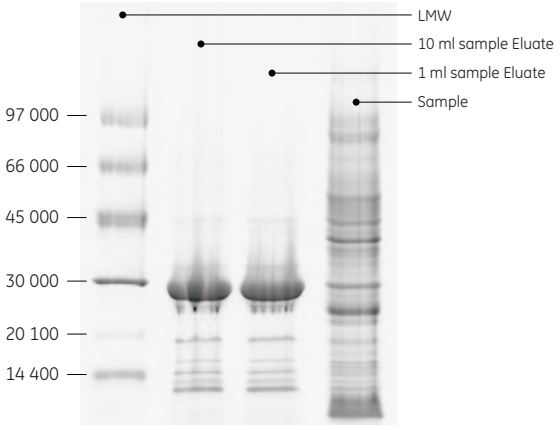

Fig 7. SDS-PAGE stained with Deep Purple Total Protein Stain. The purity obtained was equally high when scaling up the purification ten times.

Ordering Information

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

Related products	Quantity	Code No.
MagRack 6	1	28-9489-64
His Buffer Kit	1	11-0034-00
HiTrap Desalting	5 × 5 ml	17-1408-01
PD MiniTrap G-25	50 columns	28-9180-07
Protein A Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-06
Protein A Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-78
Protein A Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-56
Protein A Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-62
Protein G Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-08
Protein G Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-79
Protein G Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-66
Protein G Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-70
NHS Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-09
NHS Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-80
TiO_2 Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-10
TiO_2 Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-77

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