His GraviTrap

His GraviTrap™ is a prepacked, single-use column for purification of histidine-tagged proteins by immobilized metal affinity chromatography (IMAC). The column gives fast and simple gravity-flow purifications without any need for a purification system. Large sample volumes can be applied in one go, and the histidine-tagged protein is effectively eluted in a small volume. Table 1 lists the main characteristics of His GraviTrap.

His GraviTrap contains Ni Sepharose™ 6 Fast Flow medium, which has negligible nickel leakage and is compatible with denaturing and reducing agents as well as a wide range of additives. The columns are made of biocompatible polypropylene. Special frits protect the medium from running dry during purification.

Performance benefits of His GraviTrap include:

- Short purification times, approx. 30 min
- High protein binding capacity, ≈ 40 mg/column
- Purifies unclarified samples

Table 1. His GraviTrap characteristics

Column material	Polypropylene barrel, polyethylene frits
Medium	Ni Sepharose 6 Fast Flow
Average bead size	90 µm
Protein binding capacity[1]	Approx. 40 mg histidine-tagged protein/column
Bed volume	1 ml
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents (see Table 2)
Avoid in buffers	Chelating agents, e.g. EDTA, EGTA, citrate, (see Table 2)
pH stability, short-term (2 h)[2]	2 to 14
Storage	20% ethanol
Storage temperature	4°C to 30°C

[1] Binding capacity is protein-dependent
[2] Ni^{2+}-stripped medium

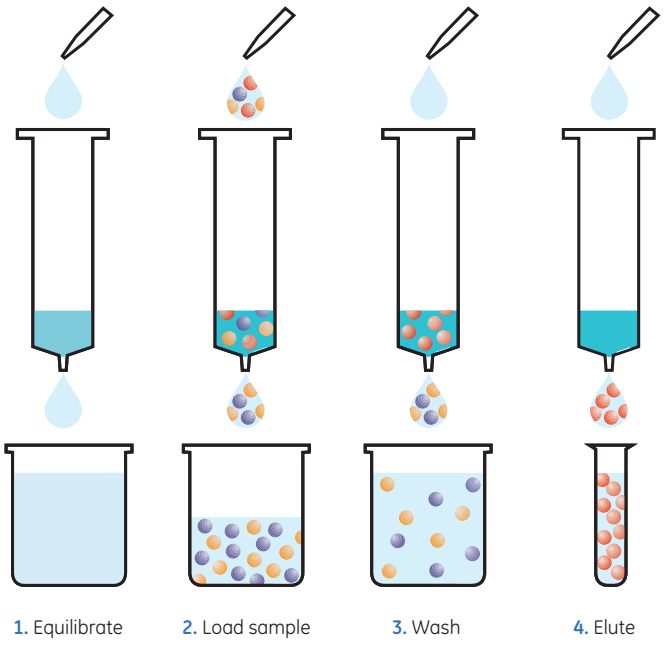


Fig 1. Purifying histidine-tagged proteins with His GraviTrap is a simple, four-stage procedure.

Operation

Purification of histidine-tagged proteins on His GraviTrap can be divided into four stages: equilibration, sample application, washing, and elution (Fig 1).

One purification run takes approximately 30 min (depending on sample volume and viscosity of the solutions).

The imidazole concentration in the sample and binding buffer influences final product purity. For His GraviTrap, we recommend 20 to 40 mM imidazole, which is somewhat higher than for similar products on the market. Elution is simply performed with 500 mM imidazole in the elution buffer. Lowering pH to pH 4.5 is an alternative for elution. Purification can be performed either under native or denaturing conditions, and a number of additives can be used (see Table 2).

Speed and convenience

His GraviTrap gives fast and simple purifications by gravity flow, a procedure that can be further improved by using the following products (Fig 2).

Workmate and LabMate

His GraviTrap columns are delivered in a package that can be converted into a column stand (Workmate). The plastic tray in this package can be used to collect liquid waste. When handling volumes above 10 ml, connecting LabMate™ reservoir to the column increases the loading capacity to approx. 35 ml in one go.

His Buffer Kit

For optimal performance and convenience, use His GraviTrap together with His Buffer Kit. His Buffer Kit contains phosphate buffer concentrates and highly pure 2 M imidazole stock solutions, thus eliminating time-consuming buffer preparation.

Fig 2. His GraviTrap connected to LabMate buffer reservoir allows loading volumes up to 35 ml in one go. For optimal performance, use buffers prepared from His Buffer Kit.

Fast purification and small elution volumes

Performance parameters to consider when running gravity columns depend on the aim of the purification. Time, elution volume, and target protein purity are all aspects to consider. In the comparative study reported below, a short total purification time and obtaining the protein in a small elution volume were the main objectives.

His GraviTrap and Ni-NTA Superflow™ gravity column (Qiagen™) were compared for purifying histidine-tagged Green Fluorescent Protein (GFP-$[His]_6$) in *E. coli* BL21 lysate.

The imidazole concentration in the sample and binding buffer was 45 mM for His GraviTrap and 10 mM for Ni-NTA Superflow (the latter according to Qiagen's recommended protocol).

The results show a clear difference regarding total purification time; 20 min with His GraviTrap and 105 min with Ni-NTA Superflow (Fig 3).

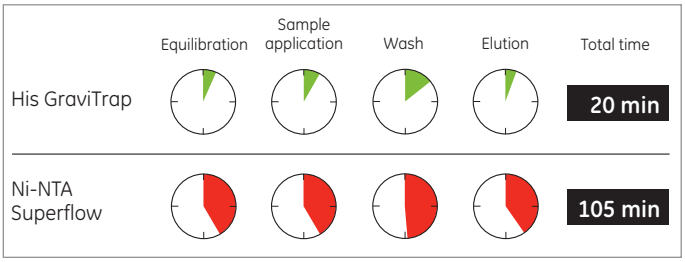


Fig 3. Comparison of total purification time for His GraviTrap and Ni-NTA Superflow gravity column under native conditions. His GraviTrap was five-times faster. All comparative studies described in this Data File were performed at GE Healthcare, Protein Separations laboratories.

Recovery during elution was > 98% in the first 3-ml eluate (eluate 1) from His GraviTrap, and approximately 80% from Ni-NTA Superflow. For complete elution from Ni-NTA Superflow, a total volume of 6 to 9 ml was needed. Recovery was calculated using absorbance measurements and extinction coefficient. The purity of eluates was similar as determined by SDS-PAGE (Fig 4).

Method:

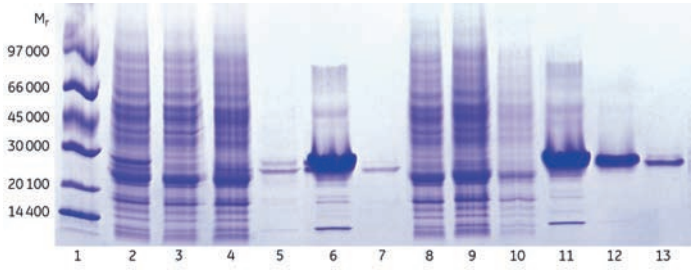
Equilibration:	10 ml binding buffer
Sample application:	10 ml clarified *E. coli* BL21 lysate containing 8 mg GFP-$(His)_6$
Wash 1:	10 ml wash buffer
Wash 2:	5 ml wash buffer
Elution 1:	3 ml elution buffer
Elution 2:	3 ml elution buffer
Elution 3:	3 ml elution buffer

Buffers His GraviTrap:

Binding and wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 45 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

Buffers Ni-NTA Superflow:

Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4
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



1. LMW markers
2. His GraviTrap, start material (diluted 1:10)
3. His GraviTrap, flowthrough (diluted 1:10)
4. His GraviTrap, wash 1
5. His GraviTrap, wash 2
6. His GraviTrap, eluate 1
7. His GraviTrap, eluate 2
8. Ni-NTA Superflow, flowthrough (diluted 1:10)
9. Ni-NTA Superflow, wash 1
10. Ni-NTA Superflow, wash 2
11. Ni-NTA Superflow, eluate 1
12. Ni-NTA Superflow, eluate 2
13. Ni-NTA Superflow, eluate 3

Fig 4. SDS-PAGE under nonreducing conditions (ExcelGel™ SDS Gradient 8–18). The eluted histidine-tagged protein is obtained in a smaller elution volume with His GraviTrap than with Ni-NTA Superflow. The minor amount of histidine-tagged protein in wash 2 (lane 5) indicates that the imidazole concentration in the His GraviTrap binding buffer was slightly too high.

High capacity and application of large sample volumes

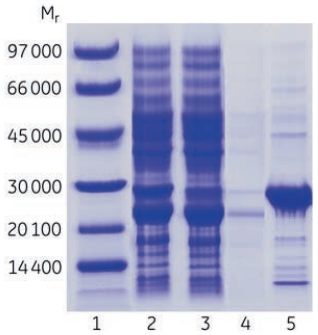
His GraviTrap columns are delivered prepacked with Ni Sepharose 6 Fast Flow. This medium displays a higher protein binding capacity compared with IMAC media from other manufacturers (see Data File 11-0008-86). In this experiment, 35-ml *E. coli* BL21 lysate containing 40-mg GFP-$(His)_6$ was applied to His GraviTrap. This large sample volume could be applied in one go since a LabMate buffer reservoir was connected to the column (Fig 2). Results show a recovery close to 100% as calculated using absorbance measurements and the extinction coefficient. Purity, as determined by SDS-PAGE (Fig 5), is comparable to earlier results when only 8 mg of sample protein was loaded (Fig 4). Total purification time was only 40 min.

Method:

Equilibration:	10 ml binding buffer
Sample application:	35 ml clarified *E. coli* BL-21 lysate containing 40-mg GFP-$(His)_6$
Wash:	20 ml binding buffer
Elution:	5 ml elution buffer

Buffers:

Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



1. LMW markers
2. Start material (diluted 1:10)
3. Flowthrough (diluted 1:10)
4. Wash (diluted 1:10)
5. Eluate (diluted 1:10)

Fig 5. SDS-PAGE under reducing conditions (ExcelGel SDS Gradient 8–18). His GraviTrap purification of 40-mg target protein applied in a sample volume of 35 ml.

Purification under denaturing conditions

His GraviTrap and Ni-NTA Superflow column (Qiagen) were compared under denaturing conditions. Histidine-tagged Maltose Binding Protein (MBP-$[His]_6$) in *E. coli* BL21 lysate containing 7 M urea was applied to the columns. Elution was performed by lowering the pH from pH 8.0 to pH 4.5.

Running under denaturing conditions increases the purification time due to the higher viscosity of the samples and buffers. Nevertheless, total purification time still was approximately four times faster on His GraviTrap; 40 min compared with 165 min for Ni-NTA Superflow (Fig 6). The purity of both eluted pools was similar as determined by SDS-PAGE (Fig 7).

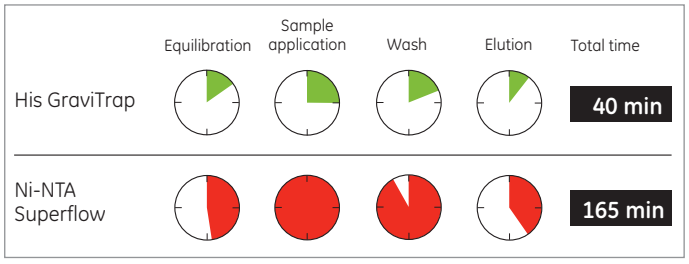


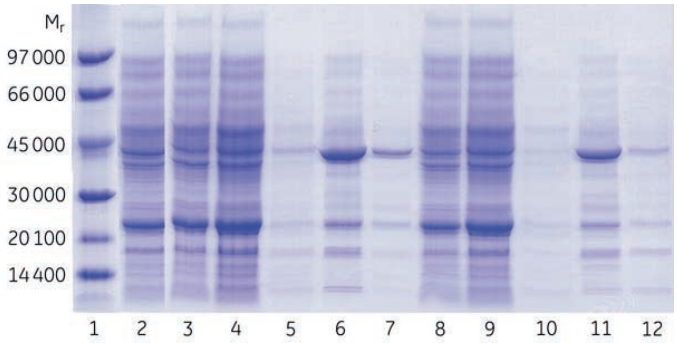
Fig 6. Comparison of total purification times for His GraviTrap and Ni-NTA Superflow gravity column under denaturing conditions. His GraviTrap was approximately four-times faster.

Method:

Equilibration:	10 ml binding buffer
Sample application:	10 ml clarified *E. coli* BL21 lysate containing 8-mg MBP-$(His)_6$
Wash 1:	10 ml binding buffer
Wash 2:	10 ml binding buffer
Elution 1:	3 ml elution buffer
Elution 2:	3 ml elution buffer

Buffers His GraviTrap and Ni-NTA Superflow:

Binding buffer:	100 mM sodium phosphate, 10 mM Tris-HCl, 7 M urea, pH 8.0
Elution buffer:	100 mM sodium phosphate, 10 mM Tris-HCl, 8 M urea, pH 4.5



1. LMW markers
2. Start material (diluted 1:20)
3. Ni-NTA Superflow, flowthrough (diluted 1:20)
4. Ni-NTA Superflow, wash 1
5. Ni-NTA Superflow, wash 2
6. Ni-NTA Superflow, eluate 1 (diluted 1:10)
7. Ni-NTA Superflow, eluate 2 (diluted 1:10)
8. His GraviTrap, flowthrough (diluted 1:20)
9. His GraviTrap, wash 1
10. His GraviTrap, wash 2
11. His GraviTrap, eluate 1 (diluted 1:10)
12. His GraviTrap, eluate 2 (diluted 1:10)

Fig 7. SDS-PAGE under reducing conditions (ExcelGel SDS Gradient 8–18). Purification on His GraviTrap and Ni-NTA Superflow results in similar purity and recovery, but the purification was four-times faster on His GraviTrap.

Purification of unclarified sample

His GraviTrap columns allow direct purification of unclarified cell lysates. The proposed procedure for preparing samples is enzymatic lysis followed by mechanical lysis, for example sonication. To reduce the viscosity of the sample, extend the duration of sonication.

Purifying unclarified samples saves time by eliminating centrifugation, which normally takes 40 to 60 min including handling tubes, etc. Avoiding centrifugation also reduces the risk of losing target protein during manual operations such as transfer to centrifugation tubes, collecting supernatant, etc. In addition, short sample preparation time generally minimizes degradation and oxidation of sensitive target proteins.

Different fermentations of *E. coli* BL21 or DH5α lysates containing GFP-$(His)_6$ were subjected to enzymatic lysis followed by extended sonication for 10 min. For comparison, one sample was also clarified by centrifugation. When volumes of 10 ml and 20 ml of unclarified sample were applied to His GraviTrap, purification time increased but the total time for sample preparation and purification was less than when clarified sample was used (Fig 8). The final purity of eluates from unclarified and clarified samples was similar as confirmed by SDS-PAGE (Fig 9).

Sample preparation and purification time

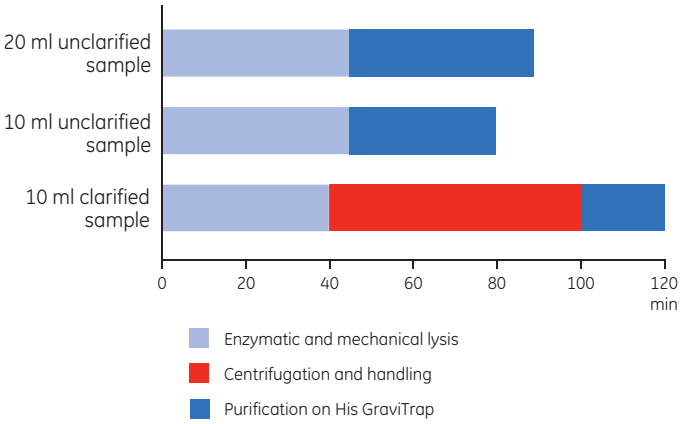


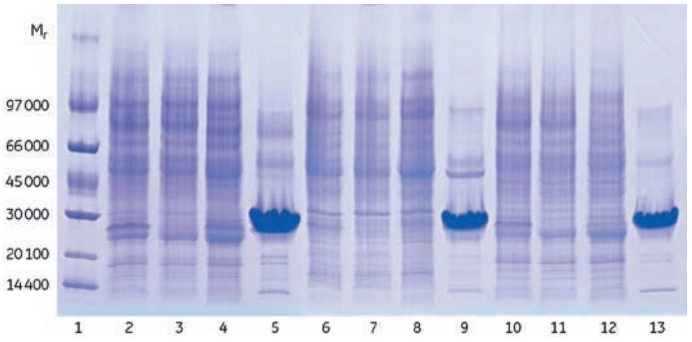
Fig 8. Total times for preparing and purifying unclarified samples are less than for clarified samples as the extra time needed to clarify the cell lysate by centrifugation is avoided.

Method:

Equilibration:	10 ml binding buffer
Sample application:	20 ml unclarified *E. coli* BL21 lysate containing 14.8 mg GFP-$(His)_6$ or 10 ml unclarified *E. coli* DH5α lysate containing 7.4 mg GFP-$(His)_6$ or 10 ml clarified *E. coli* BL21 lysate containing 7.4 mg GFP-$(His)_6$
Wash:	10 ml binding buffer
Elution:	3 ml elution buffer

Buffers:

Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 45 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



1. LMW markers
2. Unclarified sample (20 ml), start material (diluted 1:10)
3. Unclarified sample (20 ml), flowthrough (diluted 1:10)
4. Unclarified sample (20 ml), wash
5. Unclarified sample (20 ml), eluate
6. Unclarified sample (10 ml), start material (diluted 1:10)
7. Unclarified sample (10 ml), flowthrough (diluted 1:10)
8. Unclarified sample (10 ml), wash
9. Unclarified sample (10 ml), eluate
10. Clarified sample (10 ml), start material (diluted 1:10)
11. Clarified sample (10 ml), flowthrough (diluted 1:10)
12. Clarified sample (10 ml), wash
13. Clarified sample (10 ml), eluate

Fig 9. SDS-PAGE under non-reducing conditions (ExcelGel SDS Gradient 8–18). Similar purities and recoveries were obtained from unclarified and clarified samples.

High stability and compatibility

Ni Sepharose 6 Fast Flow consists of 90-µm beads of highly cross-linked agarose to which a chelating ligand has been immobilized and charged with Ni^{2+} ions. The medium is compatible with a wide range of additives commonly used in the purification of histidine-tagged proteins. Table 2 lists the compatibility of Ni Sepharose 6 Fast Flow.

Table 2. His GraviTrap is compatible with the following compounds at the concentrations given

Reducing agents*	5 mM DTE 5 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione
Denaturing agents†	8 M urea‡ 6 M guanidine-HCl‡
Detergents	2% Triton™ X-100 (nonionic) 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§ 60 mM citrate§
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, pH 4†

* For optimal performance, remove weakly bound Ni^{2+} ions with a prewash of 5 ml elution buffer. Do not leave His GraviTrap columns with buffers containing reducing agents when not in use.

† Tested for one week at 40°C.

‡ Room temperature is recommended due to the higher viscosity.

‡ 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 sample centrifugation/filtration.

Negligible nickel leakage

The ability of Ni Sepharose 6 Fast Flow to bind and hold nickel ions was tested. Low leakage was seen over a wide range of nickel capacities tested, demonstrating that the synthesis and coupling procedures used in manufacturing result in a highly homogeneous chelating ligand. In contrast, nickel leakage from Ni-NTA Superflow (Qiagen) investigated using the same test was found to be approximately twice as high as from Ni Sepharose 6 Fast Flow (Table 3). The low nickel leakage of Ni Sepharose 6 Fast Flow minimizes protein precipitation and increases yield.

Table 3. Nickel leakage test performed at pH 4.0*

Medium	Ni^{2+} leakage
Ni Sepharose 6 Fast Flow	< 5%
Ni-NTA Superflow	9%†

* Details of the comparison between Ni Sepharose 6 Fast Flow and Ni-NTA Superflow are available at www.gehealthcare.com/protocol-his. All experiments were performed at GE Healthcare, Protein Separations laboratories.

† Batch-to-batch variation was observed.

Ordering information

Designation	No. supplied	Code no.
His GraviTrap	10 x 1 ml	11-0033-99
His GraviTrap Kit *(includes 2 packs His GraviTrap and 1 pack His Buffer Kit)*	1	28-4013-51
His Buffer Kit *(includes 2 × 100 ml phosphate buffer, 8 × stock solution, pH 7.4 and 1 × 100 ml 2 M imidazole, pH 7.4)*	1	11-0034-00

Related products	No. supplied	Code no.
LabMate PD-10 Buffer Reservoir	10	18-3216-03

Literature	Code no.
Recombinant Protein Handbook, Protein Amplification and Simple Purification	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86
Ni Sepharose and IMAC Sepharose Selection Guide	28-4070-92
Data File Ni Sepharose 6 Fast Flow	11-0008-86

Acknowledgements

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