

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 FastFlow 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 Ni²⁺-stripped medium

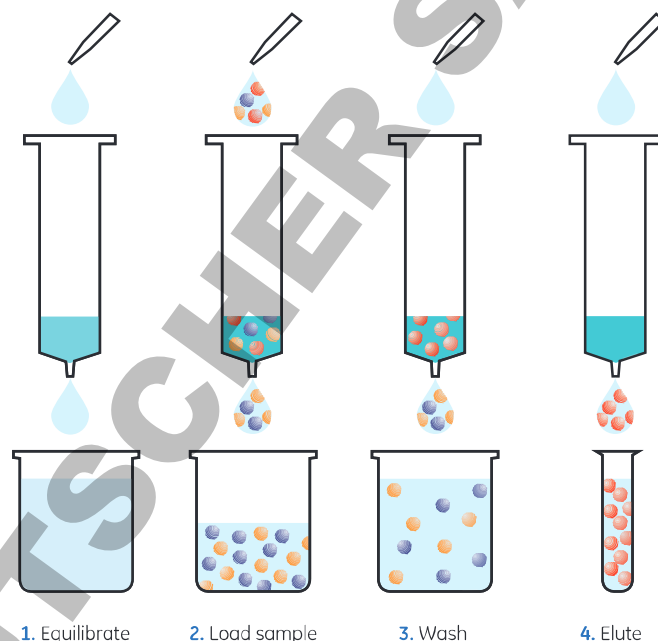


Fig 1. Purifying histidine-tagged proteins with His GraviTrap is a simple, four-stage procedure.

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).

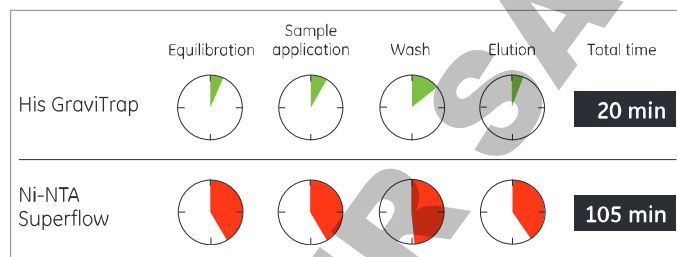


Fig 3. Comparison of total purification time for His GraviTrap and Ni-NTA Superflow gravity column under native conditions. His GraviTrap was five-times faster. All comparative studies described in this Data File were performed at GE Healthcare, Protein Separations laboratories.

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:

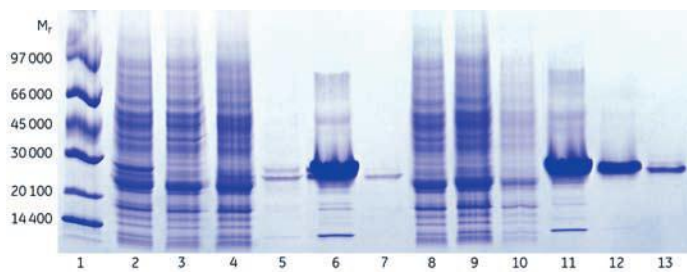
Equilibration:	10 ml binding buffer
Sample application:	10 ml clarified <i>E. coli</i> BL21 lysate containing 8 mg GFP-[His] ₆
Wash 1:	10 ml wash buffer
Wash 2:	5 ml wash buffer
Elution 1:	3 ml elution buffer
Elution 2:	3 ml elution buffer
Elution 3:	3 ml elution buffer

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

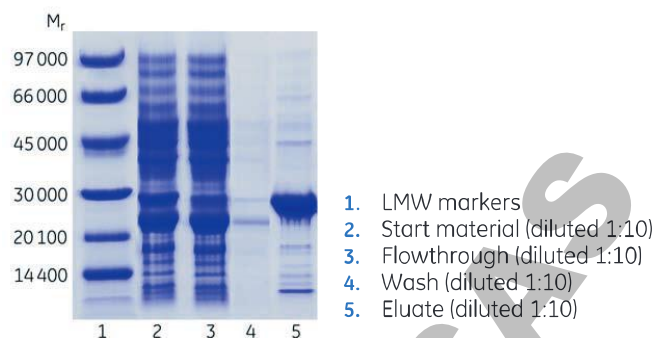
His GraviTrap columns are delivered prepacked with Ni Sepharose 6 Fast Flow. This medium displays a higher protein binding capacity compared with IMAC media from other manufacturers (see Data File 11-0008-86). In this experiment, 35-ml *E. coli* BL21 lysate containing 40-mg GFP-(His)₆ was applied to 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).

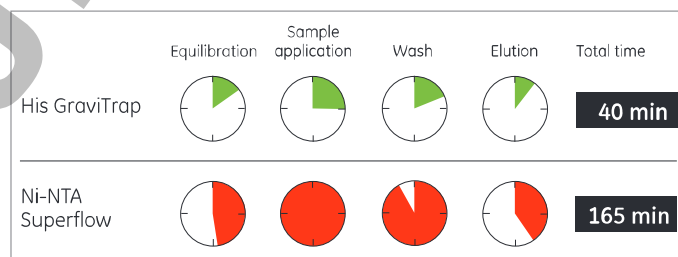


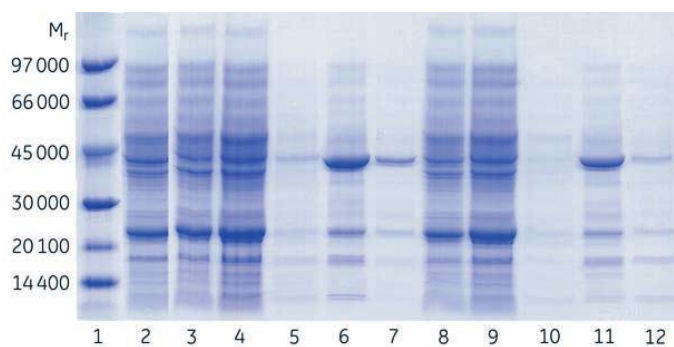
Fig 6. Comparison of total purification times for His GraviTrap and Ni-NTA Superflow gravity column under denaturing conditions. His GraviTrap was approximately four-times faster.

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.

Sample preparation and purification time

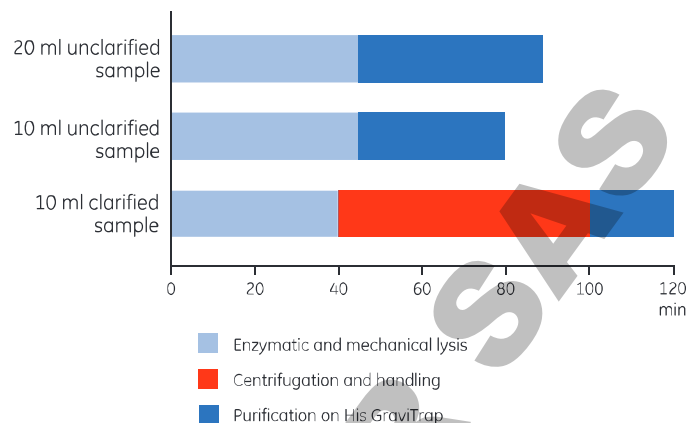


Fig 8. Total times for preparing and purifying unclarified samples are less than for clarified samples as the extra time needed to clarify the cell lysate by centrifugation is avoided.

Method:

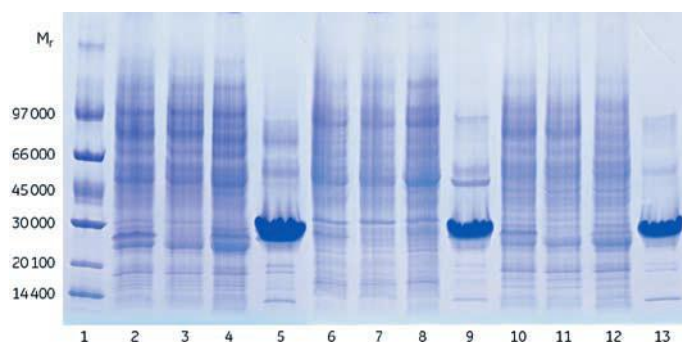
Equilibration: 10 ml binding buffer
Sample application: 20 ml unclarified *E. coli* BL21 lysate containing 14.8 mg GFP-(His)₆

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

10 ml binding buffer
 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.

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).

High stability and compatibility

Ni Sepharose 6 Fast Flow consists of 90- μ m beads of highly cross-linked agarose to which a chelating ligand has been immobilized and charged with Ni²⁺ 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 ₂ SO 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 Ni²⁺ 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 MgCl₂ 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 <i>(includes 2 packs His GraviTrap and 1 pack His Buffer Kit)</i>	1	28-4013-51
His Buffer Kit <i>(includes 2 x 100 ml phosphate buffer, 8 x stock solution, pH 7.4 and 1 x 100 ml 2 M imidazole, pH 7.4)</i>	1	11-0034-00

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

www.gehealthcare.com/his
www.gehealthcare.com

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

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

GFP-(His)₆ was provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.
MBP-(His)₆ was provided by Pharmacia Diagnostics, Uppsala, Sweden.

ExcelGel, GraviTrap, LabMate, and Sepharose are trademarks of GE Healthcare companies.
GE, imagination at work, and GE monogram are trademarks of General Electric Company

Triton is a trademark of Union Carbide Chemicals and Plastics Co. Tween is a trademark of ICI Americas Inc. Qiagen is a trademark of the Qiagen group. Superflow is a trademark of Sterogene Bioseparations Inc.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare that supplies them. General Electric Company reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation. Contact your local GE Healthcare representative for the most current information.

Protocols of the comparative studies are found at www.gehealthcare.com/protocol-his. All experiments were performed at GE Healthcare Bio-Sciences, Protein Separations laboratories.

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent 5,284,933 and US patent 5,310,663, including corresponding foreign patents (assignee: Hoffmann-La Roche Inc).

© 2006 General Electric Company - All rights reserved.

GE Healthcare Bio-Sciences AB, a General Electric Company.

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

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

GE Healthcare UK Ltd
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 Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku,
Tokyo 169-0073, Japan

Asia Pacific Tel +65 6275 1830 Fax +65 6275 1829 • Australasia Tel + 61 2 9899 0999 Fax +61 2 9899 7511 • Austria Tel 01/57606-1619 Fax 01/57606-1627 • Belgium Tel 0800 73 888 Fax 02 416 82 06 • Canada Tel 800 463 5800 Fax 800 567 1008 • Central, East, & South East Europe Tel +43 1 972720 Fax +43 1 97272 2750 • Denmark Tel 45 16 2400 Fax 45 16 2424 • Finland & Baltics Tel +358 (0)9 512 39 40 Fax +358 (0)9 512 39 439 • France Tel 01 69 35 67 00 Fax 01 69 41 96 77
Germany Tel 089 96281 660 Fax 089 96281 620 • Greater China Tel +852 2100 6300 Fax +852 2100 6338 • Italy Tel 02 27322 1 Fax 02 27302 212 • Japan Tel +81 3 5331 9336 Fax +81 3 5331 9370 • Latin America Tel +55 11 3933 7300 Fax +55 11 3933 7304 • Middle East & Africa Tel +30 210 9600 687 Fax +30 210 9600 693 • Netherlands Tel 0800 82 82 82 1 Fax 0800 82 82 82 4 • Norway Tel 815 65 555 Fax 815 65 666 • Portugal Tel 21 417 7035 Fax 21 417 3184 • Russia & other C.I.S. & N.I.S Tel +7 (495) 956 5177 Fax +7 (495) 956 5176 • Spain Tel 93 594 49 50 Fax 93 594 49 55 • Sweden Tel 018 612 1900 Fax 018 612 1910 • Switzerland Tel 0848 8028 12 Fax 0848 8028 13 • UK Tel 0800 616928 Fax 0800 616927 • USA Tel 800 526 3593 Fax 877 295 8102



imagination at work