His GraviTrap

Caution! Contains nickel, may produce an allergic reaction.

His GraviTrap™ is a prepacked, single-use gravity-flow column containing precharged Ni Sepharose™ 6 Fast Flow. The column is intended for purification of histidine-tagged proteins by immobilized metal affinity chromatography (IMAC). Ni Sepharose 6 Fast Flow has high protein binding capacity, low nickel ion (Ni^{2+}) leakage and is compatible with denaturing agents plus a wide range of additives. Both clarified and unclarified sample can be applied to the column, and special frits protect the medium from running dry during purification.

One purification run takes approximately 30 minutes (depending on sample volume and viscosity of the solutions). Table 1 lists the main characteristics of His GraviTrap.

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*	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)**	2–14
Storage	20% ethanol
Storage temperature	+4 to +30°C

* **Note:** Binding capacity is protein-dependent.

** Ni^{2+}-stripped medium.

Workmate and LabMate

His GraviTrap columns are delivered in a package that can be converted into a column stand (Workmate) to simplify purification. The plastic tray in the package can be used to collect liquid waste.

Connecting LabMate™ reservoir (Code No. 18-3216-03) to the column increases convenience when handling volumes above 10 ml. This raises the loading capacity to approx. 35 ml in one go.

Recommended buffers

Recommended buffers for native conditions can easily be prepared from His Buffer Kit (Code No. 11-0034-00) or according to the description in Appendix A.

Native conditions:

Binding 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

Denaturing conditions:

Binding buffer: 20 mM Tris-HCl, 8 M urea, 500 mM NaCl, 5 mM imidazole, pH 8.0 + 1 mM β-mercaptoethanol

Elution buffer: 20 mM Tris-HCl, 8 M urea, 500 mM NaCl, 500 mM imidazole, pH 8.0 + 1 mM β-mercaptoethanol

The optimal concentration of imidazole needed in the sample and buffer to obtain the best purity and yield differs from protein to protein. Under native conditions, 20–40 mM imidazole in the binding buffer is suitable for many proteins. 500 mM imidazole in the elution buffer is most often sufficient to completely elute the target protein.

Note: As an alternative to elution with imidazole, you can lower the pH to approx. pH 4.5 (note that metal ions will be stripped off the medium below pH 4.0).

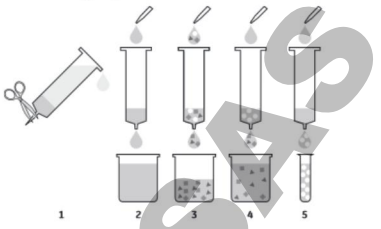
Sample preparation

The protocol below has been used successfully in our own laboratories, but other established procedures may also work.

1. **Dilute the cell paste:** Add 5–10 ml of binding buffer for each gram of cell paste. It is essential that the sample and binding buffers contain the same concenctration of imidazole to prevent binding of host cell proteins with exposed histidines.
2. **Enzymatic lysis:** 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF (final concentrations). Stir for 30 minutes at room temperature or +4°C depending on the sensitivity of the protein.
3. **Mechanical lysis:** Sonication, homogenization, repeated freeze/thaw or similar techniques.
4. **Adjust the pH of the lysate to pH 7.4:** Do not use strong bases or acids for pH-adjustment (precipitation risk).
5. **Centrifuge the lysate:** Transfer to tubes and centrifuge at 39 000 g for 20 minutes at room temperature or +4°C depending on the sensitivity of the protein.
6. **Collect supernatants and perform the purification.**

Note: You can also apply unclarified sample to the column (i.e. omitting step 5 above). If so, extend the mechanical lysis somewhat, e.g. sonicate for 10 minutes. Total purification time will increase due to the higher viscosity of the unclarified sample.

Performing a purification



1. Cut off the bottom tip, remove the top cap, pour off excess liquid and place the column in the Workmate column stand. If needed, mount LabMate on top of the column.
2. Equilibrate the column with 10 ml binding buffer. The frits protect the column from running dry during the run.
3. Add the sample (see Sample preparation). A volume of 0.5–35 ml is recommended. The protein binding capacity of the column is high, approx. 40 mg histidine-tagged protein/column (protein-dependent).
4. Wash with 10 ml binding buffer.
5. Apply 3 ml elution buffer and collect the eluate. Under denaturing conditions, elute with 2 × 3 ml elution buffer.

Note: If you use buffers containing denaturing agents or viscous solutions, perform the purification at room temperature.

Note: For purification under reducing conditions, pre-wash the column with 5 ml elution buffer and 5–10 ml binding buffer, both without the reducing agent, **before** the final equilibration.

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 (Tris[2-carboxyethyl]phsphine) 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 any weakly bound Ni^{2+} ions by performing a pre-wash as described under "Performing a purification" above. Do not leave His GraviTrap columns with buffers containing reducing agents when not in use.

** Use at room temperature due to the higher viscosity.

† Tested for one week at +40°C.

‡ 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 centrifugation/filtration of the sample.

GraviTrap

Troubleshooting

Fault	Possible cause	Action
Flow rate is too slow	The sample is too viscous	If the purification has been performed at +4°C, change to room temperature if possible. Increase dilution of the cell paste before sonication or dilute after sonication. Continue sonication until the viscosity is reduced, and/or add an additional dose of DNAse and Mg^{2+}. Filter the sample (or centrifuge if unclarified sample has been used). If very viscous solutions are used, connect the column to a vacuum manifold to speed up the flow rate.
	Target protein is difficult to dissolve or precipitates during purification	Add detergents, reducing agents or other additives (see Table 2) and mix gently for 30 minutes to aid solubilization of the tagged protein. Note that Triton X-100 and NP-40 (but not Tween) have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange. Inclusion bodies: the protein can usually be solubilized (and unfolded) from inclusion bodies using common denaturants such as 4–6 M guanidine-HCl, 4–8 M urea, or strong detergents. Mix gently for 30 minutes or more to aid solubilization of the tagged protein.
Low yield of histidine-tagged protein	Histidine-tagged protein is not completely eluted in 3 ml	Elute with an additional 3 ml of elution buffer.
	Histidine-tagged protein found in the flow-through during sample application and wash	Imidazole concentration in the sample and binding buffer is too high. Use a lower concentration. Ensure that the concentration of chelating or strong reducing agents in the sample is not too high. The histidine tag may be insufficiently exposed; perform purification of unfolded protein in urea or guanidine-HCl as for inclusion bodies. To minimize dilution of the sample, add solid urea or guanidine-HCl. The histidine tag has been lost. Check the sequence of the construct.
	Histidine-tagged protein is not eluted during purification	Histidine-tagged protein still bound. Elute with a higher concentration of imidazole in the elution buffer. The target protein has precipitated in the column. Decrease the amount of sample. Decrease imidazole concentration during elution. Try detergents or change NaCl concentration, or elute under denaturing (unfolding) conditions. Non-specific hydrophobic or other interaction. Add a non-ionic detergent to the elution buffer or increase NaCl concentration.
Eluted histidine tagged protein is not pure	Too low imidazole concentration in sample and binding buffer	Use a higher imidazole concentration in sample and binding buffer to prevent binding of unwanted host cell proteins. 20–40 mM is recommended, but higher concentrations may also be appropriate.
	Washing of unbound material is insufficient	Repeat the wash step after sample application to obtain optimal purity.
	Partial degradation of tagged protein by proteases	Add protease inhibitors (use EDTA with caution, see Table 2). Perform lysis and purify at +4°C.
	Contaminants are associated with tagged proteins	Add detergent and/or reducing agents before sonicating the cells. Increase detergent levels (e.g. up to 2% Triton X-100 or 2% Tween), or add glycerol (up to 50%) to the wash buffer to disrupt non-specific interactions.

Appendix A

2 M imidazole stock solution

To 136.2 g imidazole, add distilled water to 800 ml and dissolve completely. Adjust the pH from basic to 7.4 with HCl. Add distilled water to 1000 ml. Use high purity imidazole as this will give no or very low absorbance at 280 nm (imidazole, 68.08 g/mol).

Phosphate buffer (containing imidazole for binding and elution buffers)

(20 mM sodium phosphate, 500 mM NaCl, 10–500 mM imidazole in 1 liter)

To 1.78 g $Na_2HPO_4 \times 2H_2O$ (177.99 g/mol), 1.38 g $NaH_2PO_4 \times H_2O$ (137.99 g/mol) and 29.22 g NaCl (58.44 g/mol), add X ml 2 M imidazole stock solution (see table below). The volume of imidazole stock solution added depends on the chosen imidazole concentration during binding and elution. Finally, add distilled water to 900 ml and dissolve completely.

Adjust the pH from basic to 7.4 with HCl. Add distilled water to 1000 ml and filter through a 0.45 µm filter.

Imidazole concentration in buffer (mM)	Volume of imidazole stock solution in phosphate buffer (ml)
10	5
20	10
30	15
40	20
50	25
60	30
70	35
80	40
90	45
100	50
200	100
300	150
400	200
500	250

Ordering information

Designation	No. supplied	Code No.
His GraviTrap	10 × 1 ml	11-0033-99
His GraviTrap Kit	1	28-4013-51
(Includes 2 packs His GraviTrap and 1 pack His Buffer Kit)		

Related products	No. supplied	Code No.
LabMate PD-10 Buffer Reservoir	10	18-3216-03
His Buffer Kit	1	11-0034-00
(Includes 2 × 100 ml phosphate buffer, 8 × stock solution, pH 7.4 and 1 × 100 ml 2 M imidazole, pH 7.4)		

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

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