Fast and simple purification of histidine-tagged proteins using His GraviTrap

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Introduction

Immobilized metal ion affinity chromatography (IMAC) is a widely-used and highly effective technique that exploits the affinity of histidine-tagged proteins to bind nickel ions attached to chelating separation media such as Ni Sepharose™ 6 Fast Flow.

Histidine-tagged proteins can be quickly and simply purified on His GraviTrap™ columns prepacked with Ni Sepharose 6 Fast Flow without the need for a pump or purification system.

A single column allows purification of approx. 40 mg protein in as little as 20 minutes. Large volumes of clarified or unclarified samples can easily be applied and the purified protein can be eluted in a small volume resulting in a highly concentrated target protein.

Conclusions

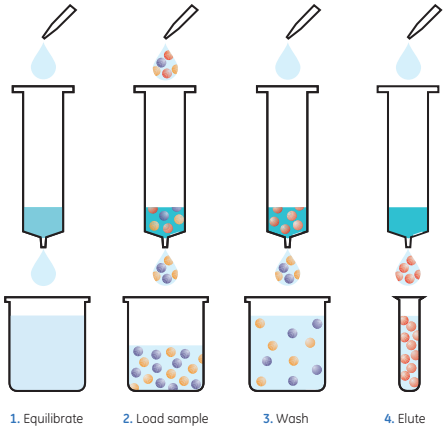
His GraviTrap gives the following performance benefits:

- Five-times faster purification than the comparable product from another supplier.
- Possibility to purify both clarified and unclarified samples.
- High protein binding capacity, approx. 40 mg/column.
- No instrumentation, e.g. pump, needed.

GraviTrap™

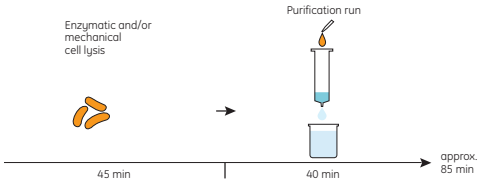
Operation

The purification of histidine-tagged proteins on His GraviTrap can be divided into four stages: equilibration, sample application, washing and elution.

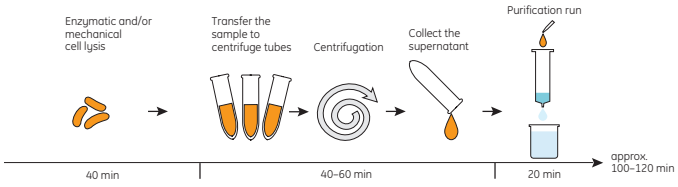


Purification of unclarified sample

Unclarified sample



Clarified sample



Purification of unclarified samples

- saves time
- reduces the risk of loosing target protein during manual handling
- minimizes degradation and oxidation of sensitive target proteins

Materials

Purification conditions, unless otherwise stated, were:

Columns:	His GraviTrap 1 ml Ni-NTA Superflow™ gravity column 1.5 ml (Qiagen™)
Samples:	Cell extracts from different strains of *E. coli* containing 0.5 M NaCl and imidazole at a concentration appropriate for each target protein.
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, × mM imidazole, pH 7.4 (× = optimized for each target protein)
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

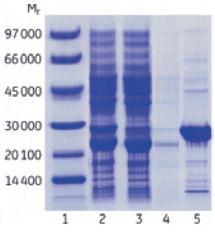
SDS-PAGE was performed with ExcelGel™ SDS Gradient 8–18 Gels.

High protein binding capacity

Sample: 35 ml clarified *E. coli* BL-21 lysate containing 40 mg GFP-$(His)_6$

Method:

Equilibration:	10 ml binding buffer (5 mM imidazole)
Sample application:	35 ml sample (5 mM imidazole)
Wash:	20 ml binding buffer (5 mM imidazole)
Elution:	5 ml elution buffer



1. Low molecular weight markers
2. Start material (diluted 1:10)
3. Flow-through (diluted 1:10)
4. Wash (diluted 1:10)
5. Eluate (diluted 1:10)

Results

- Recovery of GFP-$(His)_6$ was approx. 100 % as calculated using absorbance measurements and the extinction coefficient.
- Purity was comparable with results when just 8 mg sample protein was loaded (see the following experiment).
- The total purification time when 35 ml sample was applied was only 40 minutes

Fast purification and small elution volumes

Sample: 10 ml clarified *E. coli* BL-21 lysate containing 8 mg GFP-$(His)_6$

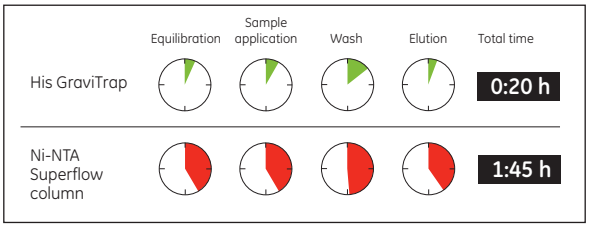
Method:

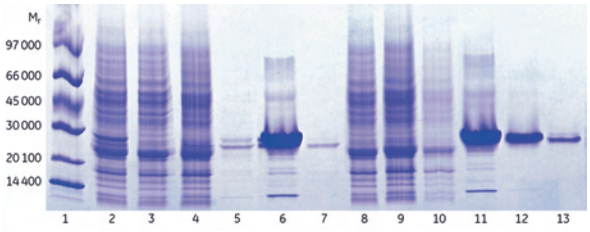
His GraviTrap:

Equilibration:	10 ml binding buffer (45 mM imidazole)
Sample application:	10 ml sample (45 mM imidazole)
Wash 1:	10 ml binding buffer (45 mM imidazole)
Wash 2:	5 ml binding buffer (45 mM imidazole)
Elution:	3 × 3 ml elution buffer

Ni-NTA Superflow:

Equilibration:	10 ml binding buffer (10 mM imidazole)
Sample application:	10 ml sample (10 mM imidazole)
Wash 1:	10 ml binding buffer (20 mM imidazole)
Wash 2:	5 ml binding buffer (20 mM imidazole)
Elution:	3 × 3 ml elution buffer





His GraviTrap

1. Low molecular weight markers
2. Start material (diluted 1:10)
3. Flow-through (diluted 1:10)
4. Wash 1
5. Wash 2
6. Eluate 1
7. Eluate 2

Ni-NTA Superflow

8. Flow-through (diluted 1:10)
9. Wash 1
10. Wash 2
11. Eluate 1
12. Eluate 2
13. Eluate 3

Results

- Total purification time for His GraviTrap under native conditions was five-times faster than with Ni-NTA Superflow.
- Recovery during elution, calculated using absorbance measurements and extinction coefficient, 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 6–9 ml was needed.
- The purity of the eluates was similar.

Rapid purification of a high molecular weight Histidine-tagged protein

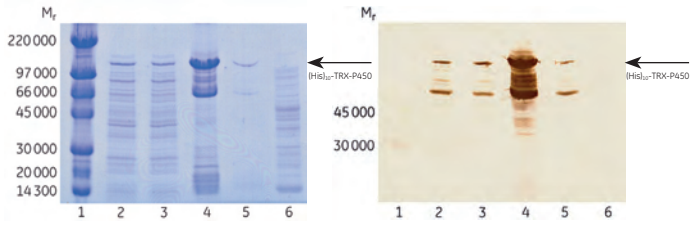
Sample: 20 ml clarified *E. coli* JM109 lysate containing $(His)_{10}$-TRX-P450 (M_r~ 130 000)

Method:

Equilibration:	10 ml binding buffer (40 mM imidazole)
Sample application:	20 ml sample (40 mM imidazole)
Wash:	2 × 10 ml binding buffer (40 mM imidazole)
Elution:	2 × 3 ml elution buffer

Western Blot:

Electrophoresis and transfer:	PhastSystem™ and PhastGel™ Gradient 10–15
Membrane:	Hybond™ ECL
Primary antibody:	Anti-His antibody (mouse)
Secondary antibody:	Anti-mouse IgG, HRP-linked
Detection:	Colorimetric, DAB-enhanced liquid substrate



1. High-Range Rainbow Molecular Weight Markers
2. Start material (diluted 1:20)
3. Flow-through (diluted 1:20)
4. Eluate 1
5. Eluate 2
6. Negative control, (JM109 non-transformed)

Results

- SDS-PAGE analysis shows three major protein bands in the eluted fractions
- Western blot analysis and N-terminal sequencing (data not shown) confirm that each of the three bands in the eluates contains a histidine tag. The low molecular weight bands are truncated forms of the histidine-tagged target protein.
- The whole purification took just 25 minutes.

Acknowledgement

We thank the owner of the clone used in this work: GFP-$(His)_6$ was provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University.

www.amershambiosciences.com/his

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Protocols for the comparative studies described are found at www.amershambiosciences.com/protocol-his

All experiments were performed at GE Healthcare Bio-Sciences, Protein Separations laboratories.

Amersham Biosciences AB, a General Electric Company going to market as GE Healthcare.

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

imagination at work

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