WorkBeads NiMAC
BabyBio NiMAC 1 ml
BabyBio NiMAC 5 ml

WorkBeads™ NiMAC resin and BabyBio™ NiMAC prepacked columns allow quick and easy purification of His-tagged proteins and other proteins with an affinity for nickel ions. WorkBeads NiMAC resin is precharged with very strongly bound nickel ions resulting in very high resistance to reducing agents such as DTT and chelating substances such as EDTA. The high stability allows purification of proteins from sources such as eukaryotic cell extracts, that normally would cause significant nickel ion stripping from the resin. This reduces the need for sample pre-treatment. The resin provides high purity and binding capacity and the possibility to use high flow rates for minimized process time.

- Resin with extra strongly bound Ni^{2+} resulting in extremely low nickel ion leakage
- Highly resistant to reducing agents up to 20 mM DTT
- Highly resistant to chelating substances present in eukaryotic extracts or up to 20 mM EDTA
- Very high protein binding capacity, higher than 40 mg/ml resin
- High purity and reproducible results

Resin desciption

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and high mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology purifications, from research to production scale, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids and carbohydrates. WorkBeads resins are designed for separations requiring optimal capacity and purity.

WorkBeads NiMAC resin is immobilized with chelating groups that bind nickel ions very tightly. The Ni^{2+}-charged resin binds His-tagged proteins but has unusually low affinity for other host proteins that tend to bind other resins made for Immobilized Metal Ion Affinity Chromatography (IMAC). The very tightly bound nickel ions result in extremely low nickel ion leakage. This property makes WorkBeads NiMAC excellent for purification of His-tagged proteins from large feed volumes containing chelating substances. Typical sources of this problem are extracts from eukaryotic cells, e.g. insect cells, that commonly contain reducing agents added during the extraction. WorkBeads NiMAC resin cannot be stripped of the Ni^{2+} ion and recharged.

The main characteristics of WorkBeads NiMAC resin are shown in Table 1. For more details, see instruction IN 40 653 010.

BabyBio description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from low-protein-binding polyethylene. The filters in the top and the bottom of the column have a pore size optimized to allow loading of semi-crude feed with minimal clogging.

The ready-to-use BabyBio prepacked columns are delivered with a plug in the inlet, a cut-off outlet and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10 - 32) for 1/16" o.d. tubing (standard HPLC PEEK tubing), with a female and a male connection at the top and bottom respectively.
BabyBio columns can be connected in series providing a convenient way to perform smaller scale-up experiments.

The main characteristics of BabyBio NiMAC columns are shown in Table 2. For additional information, see instruction IN 45 655 040.

Table 1. Main characteristics of WorkBeads NiMAC resin.

	WorkBeads NiMAC
Target substance	His-tagged proteins
Matrix	Highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm
Precharged ions	Nickel (II) ions, Ni^{2+}
Static binding capacity	> 80 mg/ml resin
Dynamic binding capacity[2]	> 40 mg/ml resin
Metal ion capacity[3]	> 60 µmol Cu^{2+}/ml resin
Max. flow rate (20 cm bed height and 5 bar)[4]	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and additives such as 20 mM Na_2-EDTA, 20 mM dithiothreitol (DTT), 20 mM TCEP, 20 mM β-mercaptoethanol, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 500 mM imidazole, 30% isopropanol, 0.5 M NaOH
pH stability	3 – 9 (working range) 2 – 14 (cleaning-in-place)
Storage	2 to 25 °C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Binding capacity may vary depending on protein characteristics and on flow rate used. A lower flow rate usually increases the dynamic binding capacity.
3. Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.
4. Optimal flow rate during binding is depending on the sample.

Table 2. Main characteristics of BabyBio NiMAC 1 ml and BabyBio NiMAC 5 ml columns.

	BabyBio NiMAC
Target substance	His-tagged proteins
Matrix	WorkBeads NiMAC
Column volume	1 ml 5 ml
Column dimension	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate[1]	
BabyBio NiMAC 1 ml	0.25 - 1 ml/min (37 - 150 cm/h)
BabyBio NiMAC 5 ml	1.25 - 5 ml/min (56 - 225 cm/h)
Max flow rate[2]	
BabyBio NiMAC 1 ml	5 ml/min (780 cm/h)
BabyBio NiMAC 5 ml	20 ml/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and additives such as 20 mM Na_2-EDTA, 20 mM dithiothreitol (DTT), 20 mM TCEP, 20 mM β-mercaptoethanol, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 500 mM imidazole, 30% isopropanol, 0.5 M NaOH
pH stability	3 – 9 (working range) 2 – 14 (cleaning-in-place)
Storage	2 to 25 °C in 20% ethanol

1. Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 ml/min and 5 ml/min can be used for 1 ml and 5 ml columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
2. Maximum flow rate for aqueous buffers at 20 °C. Decrease the maximum flow rate if the liquid has a higher viscosity.
Higher viscosities can be caused by low temperature or presence of additives. Use half of the maximum flow rate for 20% ethanol for example.

Application principle

WorkBeads NiMAC uses the Immobilized Metal Ion Affinity Chromatography (IMAC) technique. IMAC utilizes the affinity of histidine, cystein and tryptophan amino acid side chains on the protein surface for transition metal ions, such as Ni^{2+}, immobilized (via a metal chelating ligand) on the chromatography resin.
IMAC is most commonly used for the purification of recombinant His-tagged proteins. This His-tag is usually composed of six to ten histidyl groups and is typically placed at the N- or C-terminus of the target protein, although other positions are possible. The His-tagged proteins will bind to the chelating ligand (through the metal ion) and the unbound material will pass through.

Purification of His_6-GFP from *E. coli* extract in the presence of reducing agent and chelator

His_6-GFP was purified from an *E. coli* extract expressing His_6- Green Fluorescent Protein (His_6-GFP) (Fig. 1) using a prepacked BabyBio NiMAC 1 ml column. The extract was clarified and supplemented with 20 mM DTT and 20 mM Na_2-EDTA before 10 ml was loaded onto a column equilibrated with 10 column volumes (CV) of binding buffer containing 10 mM imidazole. After sample application, the column was washed with 10 CV of binding buffer to remove unbound impurities. The target protein was then eluted using a 25 CV gradient from 10 to 300 mM imidazole. No significant amount of His_6-GFP was detected in the flow through (or in the wash). The purity of the eluted main peak was 89%, and no impurity peaks are seen in the chromatogram. The emission maximum for His_6-GFP is 490 nm.

WorkBeads NiMAC vs. WorkBeads Ni-NTA (conventional IMAC resin)

Purification of His-tagged proteins from feeds containing reducing agents, such as DTT or chelating agents, such as EDTA, causes stripping of nickel ions from conventional IMAC resins. To analyse the difference between WorkBeads NiMAC resin and a conventional resin, comparative experiments were performed using BabyBio 1 ml columns (Fig. 2).

Aliquots of 10 ml of clarified *E. coli* extract containing His_6- GFP with 20 mM EDTA and 20 mM DTT were applied to BabyBio NiMAC 1 ml and BabyBio Ni-NTA 1 ml, the latter being a conventional column for His-tagged protein purification. The target His-tagged protein was eluted stepwise using 300 mM imidazole. Imidazole at a concentration of 10 mM was included in both sample and binding buffer to reduce unspecific binding. Higher imidazole concentrations increase purity but may reduce the yield.

Column:	BabyBio NiMAC 1 ml
Sample:	10 ml His_6-GFP in binding buffer including 20 mM DTT and 20 mM Na_2-EDTA
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Elution:	Linear gradient, 0-100% elution buffer, 25 CV
Flow rates:	0.5 ml/min (78 cm/h; elution); 1 ml/min (loading)

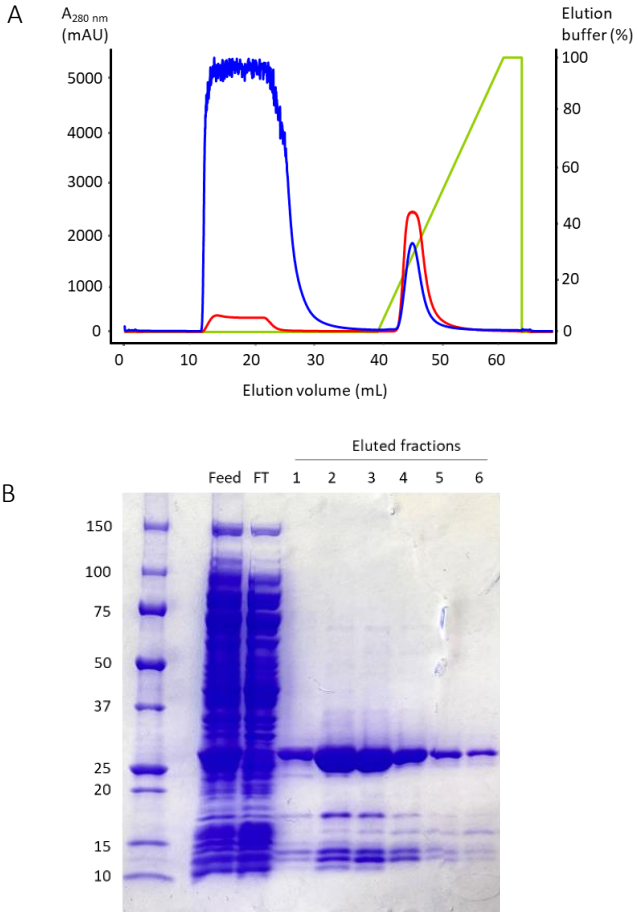


Figure 1. Purification of His_6-GFP from clarified *E. coli* extract using BabyBio NiMAC 1 ml column. (A) Chromatogram showing UV absorbance at 280 nm (blue) and 490 nm (red), and percentage of elution buffer (green). (B) SDS-PAGE analysis. Marker, feed, flowthrough (FT) and eluted peak fractions of 1.5 ml (lanes 1-6).

Columns:	BabyBio NiMAC 1 ml and BabyBio Ni-NTA 1 ml
Sample:	10 ml His_6-GFP in binding buffer including 20 mM DTT and 20 mM Na_2-EDTA
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Elution:	Step gradient, 100% elution buffer, 5 CV
Flow rates:	0.5 ml/min (78 cm/h; elution); 1 ml/min (loading)

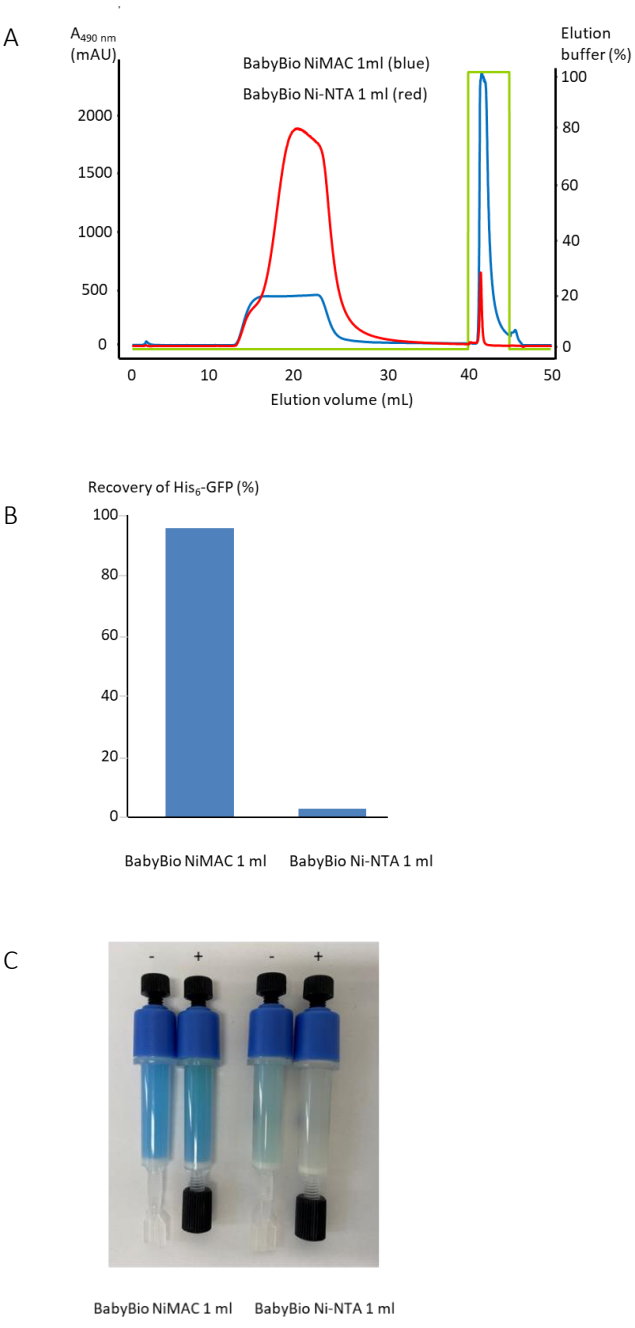


Figure 2. Purification of clarified His_6-GFP using BabyBio NiMAC 1 ml column compared to a conventional BabyBio Ni-NTA 1 ml. (A) Chromatogram of the capture, wash and elution of His_6-GFP expressed in *E. coli*. Absorbance at 490 nm for BabyBio NiMAC (blue) and BabyBio Ni-NTA (red). (B) Comparison of target recovery for the two columns. (C) The columns before (-) and after (+) the purification runs.

The difference in performance between the two resins is seen in Figure 2, where the nickel ions are stripped off from the conventional resin that turns white, whereas WorkBeads NiMAC has an outstanding recovery of protein (95%) and maintains its blue color. Even after 300 ml sample load containing 20 mM EDTA and 20 mM DTT the blue color was maintained.

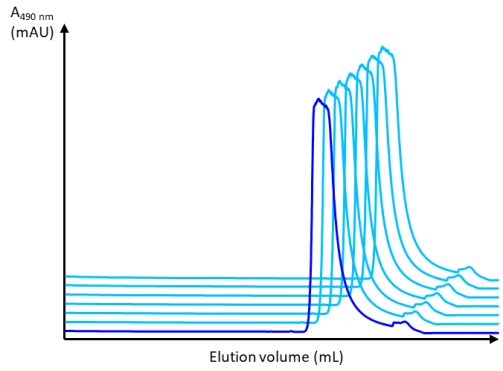
Multiple runs

The advantage of using WorkBeads NiMAC when purifying His-tagged proteins from a cell culture medium containing e.g. reducing agents that often cause metal stripping is visualized in Figure 2. But it is also of high importance that resins can be used for multiple runs without losing performance, for example binding capacity, even when large amounts of reducing agents and chelators are included in the sample and/or buffers.

To show that multiple runs can be done using WorkBeads NiMAC without losing performance, seven purification cycles were run on BabyBio NiMAC 1 ml (Fig. 3). In each cycle 10 ml clarified *E. coli* extract expressing His_6-GFP was applied. The runs were repeated without any intermediate treatment of the column. The protein was eluted by a step gradient with 300 mM imidazole. Figure 3A shows the elution profiles for each run in order, with the first run shown in dark blue. The reproducibility was excellent with a recovery reduction of less than 5% over the seven consecutive purifications. The relative purity of the eluted His_6-GFP was measured by photometry as the ratio of $A_{490\ nm}/A_{280\ nm}$. The purity in percentage was calculated based on the fact that 100% pure His_6-GFP has a ratio of 2.13, as determined after polishing by size-exclusion chromatography, and the assumption that no tentative impurity from the extract shows any absorbance at A_{490nm}. Protein purity was found to be 94.2% across the purifications (Fig. 3B). Complete capture of the target protein to the resin from the feed in the loading step was confirmed by performing SEC analysis of the feed, the flowthrough and the eluate fractions (Fig. 3C).

Column:	BabyBio NiMAC 1 ml
Sample:	7 x 10 ml His_6-GFP in binding buffer with 20 mM DTT and 20 mM Na_2-EDTA
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Elution:	Step gradient, 100% elution buffer, 5 column volumes (CV)
Flow rates:	0.5 ml/min (78 cm/h; elution); 1 ml/min (loading)

A



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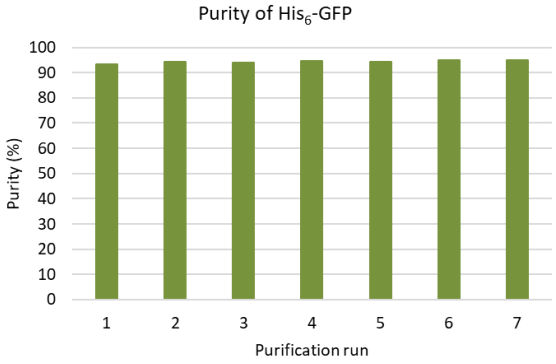

SEC analysis

Column:	Superdex™ 200 Increase 10/300 GL (Cytiva)
Sample:	60 ul flow through fraction or eluted sample
Elution buffer:	20 mM PBS, pH 7.4
Flow rate:	0.3 ml/min

C

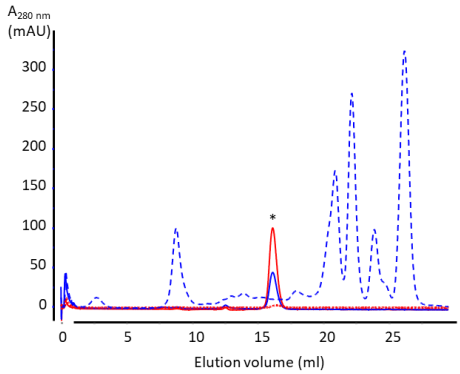

Figure 3. Seven consecutive purifications of clarified *E. coli* extract expressing His_6-GFP using a BabyBio NiMAC 1 ml column. (A) Chromatograms of the elution profile of His_6-GFP for 7 repeated runs (chromatograms shifted). Absorbance at 490 nm for the first run (blue) and consecutive runs (light blue in run order). (B) Purity measured off-line for elution pools for each purification. (C) SEC analysis of flow through fraction (dashed lines) and eluted His_6-GFP (solid lines). Absorbances in chromatogram: $A_{280\ nm}$ (blue) and $A_{490\ nm}$ (red). The asterisk highlights the His_6-GFP peak.

Comparison of reproducibility

A comparative study was performed with HisTrap™ Excel (Cytiva), another product that is tolerant against reducing agents and chelating substances. The purifications described in Fig. 3 were repeated in seven cycles also with HisTrap Excel (not shown). The recovery of His_6-GFP in run 1 was estimated to be 84% for HisTrap Excel and 95% for BabyBio NiMAC. The reduction in recovery in run 7 compared to run 1 was 5% for BabyBio NiMAC and 10% for HisTrap Excel, showing a significantly better stability of BabyBio NiMAC (Fig. 4A).

The purity was found to be 92.4% for HisTrap Excel compared with 94.2% for BabyBio NiMAC. The purity and yield are also visualized by SDS-PAGE (Fig. 4B). All these data demonstrate excellent reusability properties of WorkBeads NiMAC (Table 1).

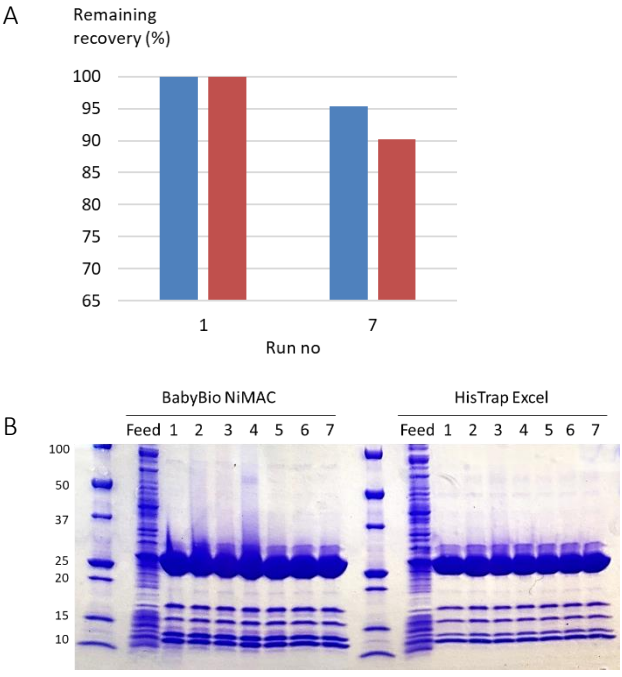

Figure 4. Seven consecutive purifications of clarified *E. coli* extract expressing His_6-GFP using a BabyBio NiMAC 1 ml column. (A) Loss of recovery for BabyBio NiMAC (blue) vs. HisTrap Excel (red) (not shown) over the 7 consecutive runs. (B) SDS-PAGE under reducing conditions of the feed and eluates from the multiple runs for both BabyBio NiMAC and HisTrap Excel.

Table 1. Data for the consecutive runs on BabyBio NiMAC and HisTrap Excel.

	BabyBio NiMAC	HisTrap Excel
Purity (average %)	94.2	92.4
Recovery (%)	95	84
Loss of recovery after seven runs (%)	4.6	9.8

Larger sample loads

The scalability of WorkBeads NiMAC was investigated by a 5-fold purification scale-up of His_6-GFP (Fig. 5).

Column:	BabyBio NiMAC 1 ml
Sample:	50 ml His_6-GFP in binding buffer with 20 mM DTT and 20 mM Na_2-EDTA
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Elution:	Step gradient, 100% elution buffer, 10 column volumes (CV)
Flow rates:	0.5 ml/min (78 cm/h; elution); 1 ml/min (loading)

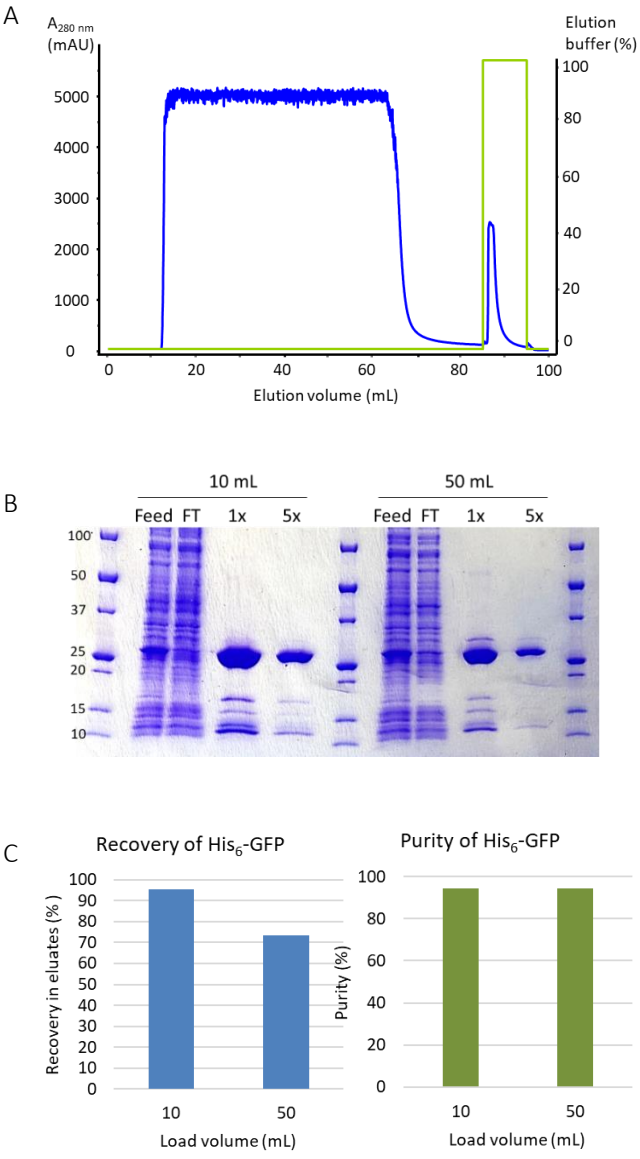

Figure 5. Large sample load on WorkBeads NiMAC (A) Chromatogram with 50 ml load of His_6-GFP eluted in 10 CV step gradient with 100% elution buffer. (B) SDS-PAGE under reducing conditions of the feed, flowthrough (FT) and eluted pool (1x: concentrated eluate, 5x: 1:5 diluted eluate) from 10 ml sample load and 50 ml sample load. (C) Comparison of target recovery and purity for the two different sample load purifications.

Purification was done by applying 50 ml *E. coli* extract expressing His_6-GFP on a BabyBio NiMAC 1 ml column (Fig. 5A). Purification was done by applying 50 ml *E. coli* extract expressing His_6-GFP on a BabyBio NiMAC 1 ml column (Fig. 5A). The purity was as good as purification using just 10 ml of feed, whereas the recovery was 73.5% for the 50 ml purification compared to 95% in the 10 ml purification (Fig. 5C).

Conclusion

WorkBeads NiMAC is an excellent choice when purifying His-tagged proteins in cell cultures containing reducing agents, such as DTT or chelating substances, such as EDTA. This particularly applies to eukaryotic cell cultures, e.g. insect cells. The EDTA concentrations used in the purification examples shown here, 20 mM, exceed the normal concentrations found in most enzyme inhibitor cocktails.

WorkBeads NiMAC resists nickel stripping from the resin and can easily be reused for multiple purification cycles with retained purity and recovery performances.

Initial wash and recharging of metal ions

When using affinity chromatography it is recommended to do an initial wash before the first usage to wash out any loosely bound ligands and/or Ni^{2+}-ions to stabilize the binding capacity over time. We recommend an initial wash of WorkBeads NiMAC with 0.5 M NaOH for 15 minutes.

It is not possible to strip and recharge WorkBeads NiMAC due to the very tight binding of the nickel ions to the chelating group immobilized on the matrix.

Cleaning-in-place (CIP)

When running complex feeds, small amounts of impurities tend to adsorb to the resin by unspecific interactions. Cleaning of resin with up to 0.5 M NaOH for 15 minutes followed by 15 minutes distilled H_2O in repeated cycles are recommended if resin gets fouled.

Storage

Store at 2 to 25 °C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio IDA His-tag Screening kit 1 ml[2]	1 ml x 4	45 700 001
BabyBio NTA His-tag Screening kit 1 ml[2]	1 ml x 4	45 700 101
Bulk resins		
WorkBeads 40 Ni-NTA	25 ml	40 651 001
WorkBeads 40 Co-NTA	25 ml	40 651 401
WorkBeads 40 Cu-NTA	25 ml	40 651 301
WorkBeads 40 Zn-NTA	25 ml	40 651 501
WorkBeads 40 Ni-IDA	25 ml	40 650 001
WorkBeads 40 Co-IDA	25 ml	40 650 401
WorkBeads 40 Cu-IDA	25 ml	40 650 301
WorkBeads 40 Zn-IDA	25 ml	40 650 501

1. Other pack sizes can be found in the complete product list on www.bio-works.com
2. Includes one column each precharged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}

Ordering information

Product name	Pack size[1]	Article number
WorkBeads NiMAC	25 ml	40 653 001
	150 ml	40 653 003
	1 L	40 653 010
BabyBio NiMAC 1 ml	1 ml x 1	45 655 311
	1 ml x 2	45 655 312
	1 ml x 5	45 655 313
	1 ml x 10	45 655 314
BabyBio NiMAC 5 ml	5 ml x 1	45 655 315
	5 ml x 2	45 644 316
	5 ml x 5	45 655 317
	5 ml x 10	45 655 318

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For more information about local distributor and products visit www.bio-works.com
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