New Ni-NTA Cartridges — the faster way to purer proteins

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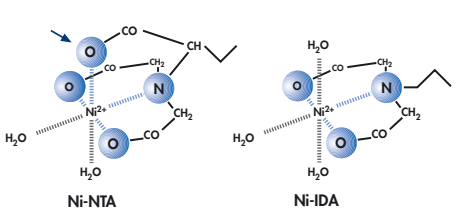
Introduction

The His-tag is the most popular and widely used affinity tag for purification of recombinant proteins. Ni-NTA matrices — the most cited material used for purification of His-tagged proteins — are available in a multitude of formats for purification on any scale. They have set the standard as the most effective method for purification of 6xHis-tagged proteins, and offer:

Ni-NTA Superflow Cartridges offer:

- Highly specific binding of 6xHis-tagged proteins — for purer preparations with fewer contaminants
- Minimal nickel leaching — reducing both nonspecific binding and the need for further cleanup
- Proven track-record in therapeutics production — support for regulatory issues available

Ni-NTA Superflow is now available in pre-filled 1 ml and 5 ml cartridges for automated purification on liquid chromatography systems such as the FPLC™ systems, or manual purification using a syringe.



The extra coordination site (arrowed) in NTA binds nickel ion more tightly than IDA (the ligand used in many competitor resins). The tighter binding means less nickel leaching and provides purer proteins.

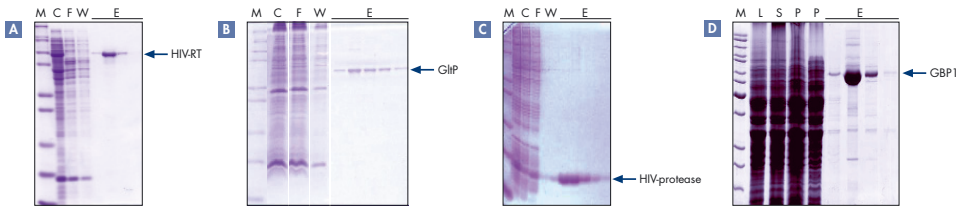
1 ml and 5 ml Ni-NTA Superflow Cartridges

The first choice for one-step purification

- Ni-NTA matrices are the affinity chromatography solution of choice for purifying 6xHis-tagged proteins.
- Their high stability means that they are compatible with a wide range of buffer components, including strong denaturants, detergents, and even reducing agents (Table 1).
- This flexibility enables researchers to develop optimal purification schemes while still benefiting from the excellent separation characteristics delivered by Ni-NTA, often making a second chromatographic step unnecessary.

Table 1. Reagents compatible with the 6xHis/Ni-NTA interaction

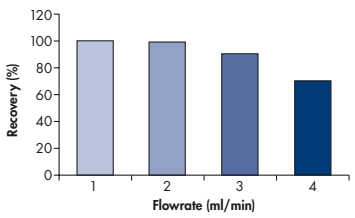
Denaturants	Detergents	Reducing agents	Others	Salts	Long-term storage
6 M GuHCl	2% Triton® X-100	20 mM β–ME	50% glycerol	4 M $MgCl_2$	Up to 30% ethanol or
8 M Urea	2% Tween®20	10 mM DTT	20% ethanol	5 mM $CaCl_2$	100mM NaOH
	1% CHAPS		20 mM imidazole	2 M NaCl	



Efficient one-step purification of His-tagged proteins using buffers containing a wide variety of additives. The indicated protein was purified in buffers containing A reducing agent (10 mM DTT) B detergent (1% n-dodecyl-β-D-maltoside) and C under denaturing conditions (8M urea). D Human GBP1 expressed in *S. cerevisiae* (data kindly provided by Julia Fres, Center for Molecular Medicine, Cologne University, Germany. D HIV-Protease. M: markers; C: cleared lysate; F: flow-through; W: wash; L: lysate; S: soluble fraction; P: pellet; E: elution fractions.

High flow rates for fast results

- The robust Superflow matrix allows flow rates up to 10 ml/min (1 ml cartridges) and 40 ml/min (5 ml cartridges), speeding the purification procedure and increasing throughput.
- The cartridges are quickly and easily connected to liquid chromatography systems or a syringe for manual purification.



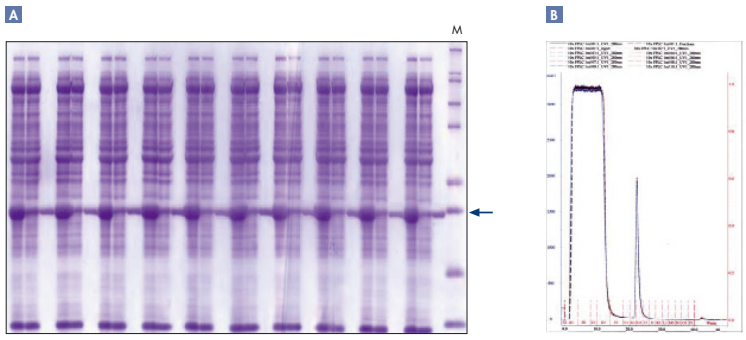
Flow rates can be increased without significant losses in yield. His-tagged GFP was purified using a 1 ml Ni-NTA Superflow Cartridge at the indicated flow rates. Relative recoveries are normalized to that obtained at 1 ml/min (100%). To speed processing, flow rates used with Ni-NTA cartridges can be increased up to 10 ml/min (1 ml cartridge) and 40 ml/min (5 ml cartridges).

Ni-NTA Cartridge Specifications

	1 ml Ni-NTA Cartridges	5 ml Ni-NTA Cartridges
Matrix	Highly cross-linked, 6% agarose	
Binding capacity	Up to 20 mg His-tagged protein	Up to 100 mg His-tagged protein
Recommended flow rate	1 ml/min	5 ml/min
Maximum flow rate	10 ml/min	40 ml/min
Maximum back pressure	0.5 MPa, 5 bar	
Column dimensions (i.d. x h)	6.7 mm x 28.0 mm	14.7 mm x 29.8 mm
pH stability (≤ 2 h)	2–14	
pH stability (≥ 2 h)	3–12	
Suitable for	FPLC, ÄKTA, and BioLogic systems, Vison workstation, HPLC instruments, or manual purification using a syringe.	
Cartridge connectors	1/16" (inlet); M 6 (outlet)	

Highly reliable and reproducible purification

- The purification process is highly reproducible, guaranteeing the same high quality protein preparations time after time.



Highly reproducible and reliable purification. A His-tagged pGAPase (arrowed) was purified in 10 sequential purification procedures. Column load was 10 ml aliquots of cleared E. coli cell lysate containing 30 mg spiked protein. Between purification runs the column was cleaned in place using 0.5 M NaOH. Groups of samples show column load, flow-through, and peak elution fraction. M: markers. B Overlaid chromatogram traces of the 10 purification runs.

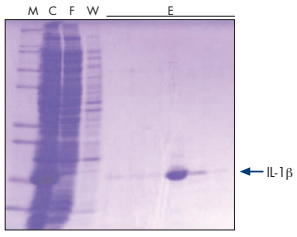
Efficient scale-up — purification of 2 grams IL-1β

- Ni-NTA Cartridges can be used to scale-up purification of His-tagged proteins for structural studies (e.g., using protein crystallography or NMR).
- As shown in Table 2, purities are consistently high over all purification scales.
- This means that if purity is high in micro-scale preparations, the same high-level of purity will be obtained in large-scale purifications

Table 2. Micro- to large-scale purification of IL-1β, for a biopharmaceutical project

Matrix	Matrix volume	Culture volume	Yield	Recovery (%)	Purity*
Ni-NTA Magnetic Agarose Beads (micro-scale)	100 µl	1 ml	33 µg	~ 90%	~ 97%
Ni-NTA Superflow (small-scale)	500 µl	320 ml	6 mg	~ 80%	~ 96%
Ni-NTA Superflow (medium-scale)	10 ml	1.7 l	109 mg	~ 80%	~ 98%
Ni-NTA Superflow (large-scale)	100 ml	18 l	2 g	> 88%	~ 97%

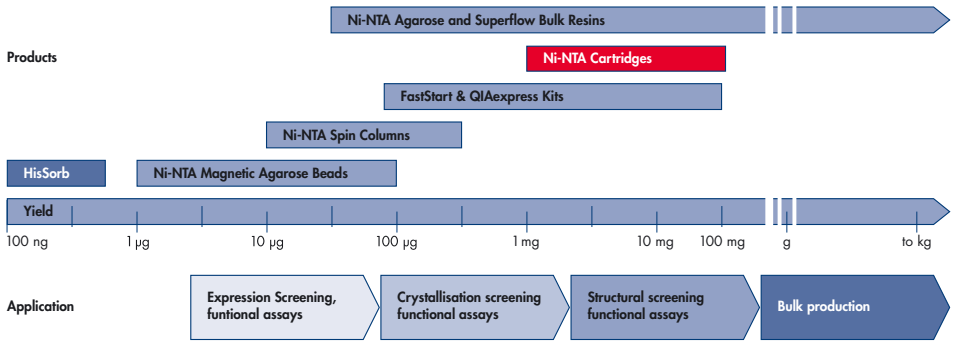
* Determined using Agilent Bioanalyzer (Protein 50 LabChip Kit)



Scaled-up expression and purification of His-tagged IL1-β that delivered a total of 2 g protein. M: markers; C: cleared lysate; F: flow-through; W: wash; L: lysate; S: soluble fraction; P: pellet; E: elution fractions.

Summary

- Ni-NTA Cartridges form part of the comprehensive and complementary solutions for His-tagged protein offered by QIAGEN.



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