Ni Sepharose 6 Fast Flow—a new high capacity nickel-charged medium for batch purification of histidine-tagged proteins

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Ni Sepharose 6 Fast Flow—a new high capacity nickel-charged medium for batch purification of histidine-tagged proteins

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Introduction

Immobilized Metal Affinity Chromatography (IMAC) is an excellent chromatography technique for purification of histidine-tagged proteins. Pure protein can be obtained from a pretreated clarified cell extract in a single step. Ni Sepharose™ 6 Fast Flow is a new medium precharged with nickel for optimized purification of histidine-tagged proteins. The medium is useful for batch/gravity-flow. Nickel leakage from the medium is negligible, which allows repeated purification of histidine-tagged proteins without recharging the medium.

Ni Sepharose 6 Fast Flow for batch purifications

- Compatible with commonly used reducing agents, such as DTT, DTE, TCEP, and β-mercaptoethanol.
- Stable in a wide range of denaturants, detergents, and buffer systems.
- Protein binding capacity higher than that found with comparative media from other manufacturers.

Compatibility with reducing and denaturing agents, detergents, additives, and buffer substances

Ni Sepharose 6 Fast Flow is stable in a wide range of reducing and denaturing agents, detergents, and additives. The medium is, for example, stable in reducing agents such as DTT at concentrations up to 5 mM (Table 1).

Table 1. Ni Sepharose 6 Fast Flow is stable in the following reducing agents, denaturing agents, detergents, additives, and buffer substances 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 Gua-HCl†
Detergents	2% Triton™ X-100 (nonionic)
	2% Tween™ 20 (nonionic)
	2% NP-40 (nonionic)
	2% cholate (anionic)
	1% CHAPS (zwitterionic)
Other additives	500 mM imidazole
	20% ethanol
	50% glycerol
	100 mM Na_2SO_4
	1.5 M NaCl
	1 mM EDTA‡
	60 mM citrate‡
Buffer substances	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†

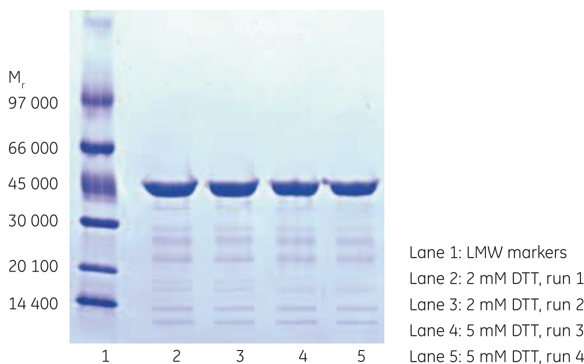
** For best results, perform a blank run before including reducing agents in the sample/buffers.*

† Tested for one week at 40 °C.

‡ The strong chelator EDTA has been used successfully in some cases at 1 mM. 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 addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

Repeated purifications of a histidine-tagged protein on the same column with buffers and sample containing 2 mM or 5 mM DTT

Column: HisTrap™ FF 1-ml
Sample: $(Histidine)_6$-tagged maltose binding protein in *E. coli* extract (conc. ~1 mg/ml, M_r ~43 000)
Sample volume: 7 ml
Binding buffer: 2 or 5 mM DTT, 20 mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4
Elution buffer: 2 or 5 mM DTT, 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4
Flow rate: 1 ml/min
A blank run without DTT was performed before the purifications.



Results:

- Purity and recovery of the protein were unaffected by DTT.

High binding capacity of Ni Sepharose 6 Fast Flow

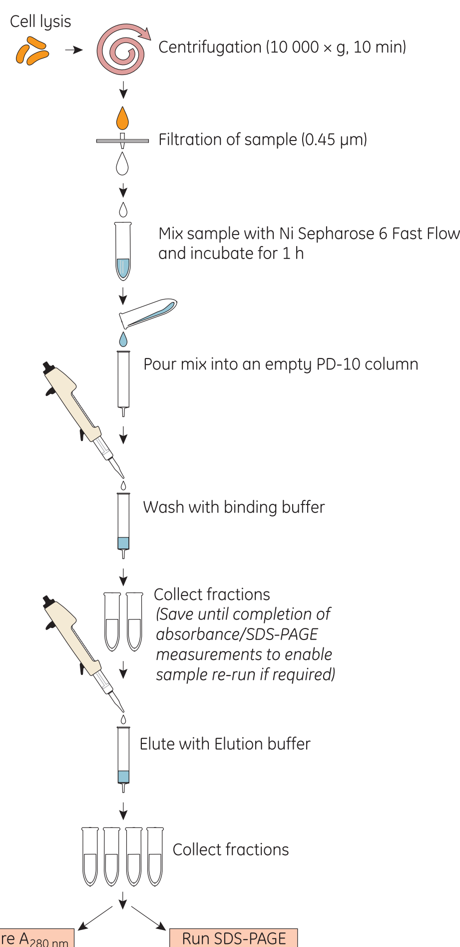
The batch purification performance of Ni Sepharose 6 Fast Flow was compared to the performance of two Qiagen™ products—Ni-NTA Superflow™ and Ni-NTA Agarose. Batch purification of histidine-tagged green fluorescent protein (GFP-$[His]_6$) was performed using empty disposable PD-10 columns packed with the different media.

Materials and methods

E. coli BL21 cells were lysed by sonication in binding buffer (20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.3). After clarification, pure GFP-$(His)_6$ was added to give 2.25 mg GFP-$(His)_6$/ml of sample.

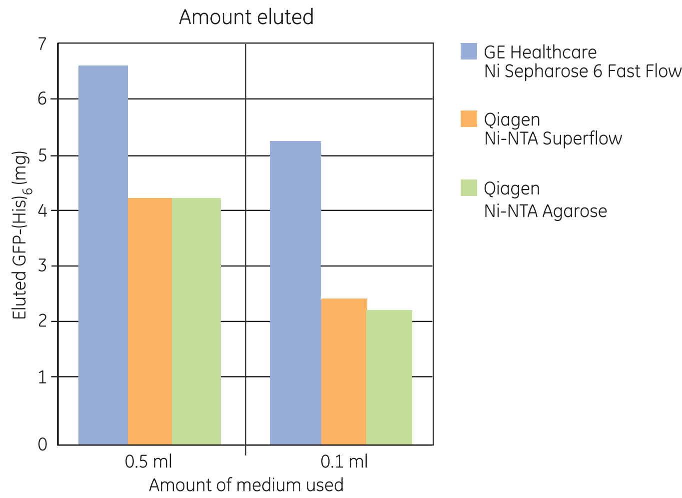
The media were prepared to give 50% slurries in binding buffer. A sample volume of 4 ml (approx. 9 mg GFP-$[His]_6$) was added to 0.2 ml or 1 ml of the 50% slurries (corresponding to 0.1 ml and 0.5 ml of the media respectively) and incubation at room temperature was performed on a shaker for 1 h. The media with bound GFP-$(His)_6$ were packed into empty disposable PD-10 columns and washed with four column volumes (CV) of binding buffer. Elution was performed using five and seven CVs of elution buffer (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.3) for 0.5 and 0.1 ml medium respectively. The amounts of eluted GFP-$(His)_6$ per ml medium were estimated by 280 nm measurements. The purity of pooled fractions was analyzed by SDS-PAGE.

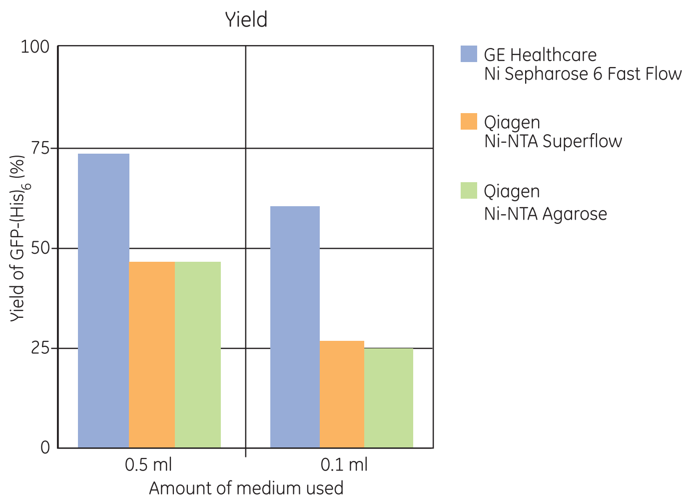
The protocol described is summarized schematically below.



Results

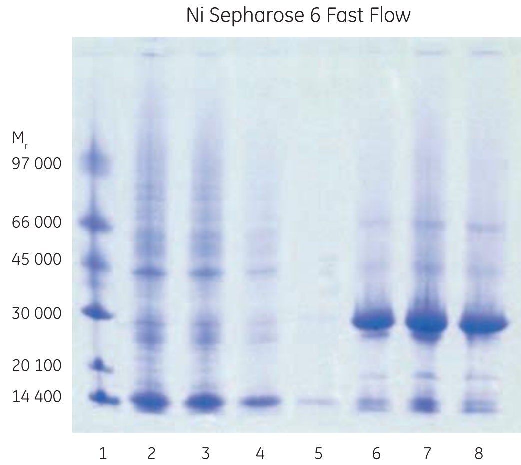
- As estimated from absorbance readings, the amount of eluted GFP-(His)$_6$ and the % yield was higher for Ni Sepharose 6 Fast Flow than for the other media.

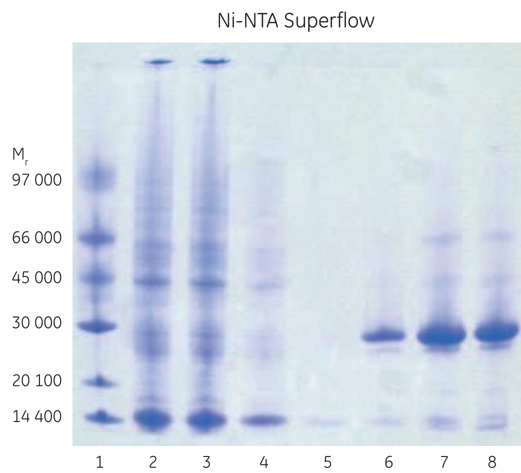


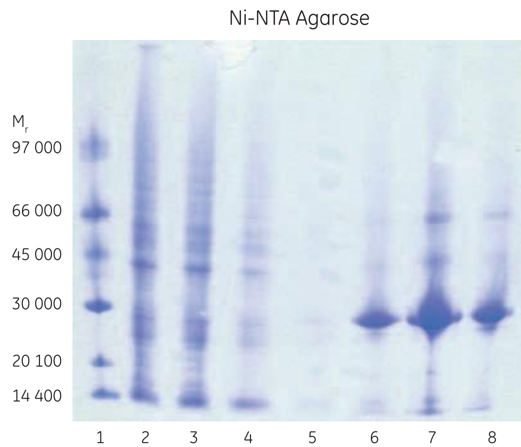


(Due to the kinetics of batch purification, the amount of GFP-(His)$_6$ was not directly proportional to the quantity of the medium used.)

- Purity of GFP-(His)$_6$ obtained with 0.1 ml medium was similar for the three media, as determined by SDS-PAGE.





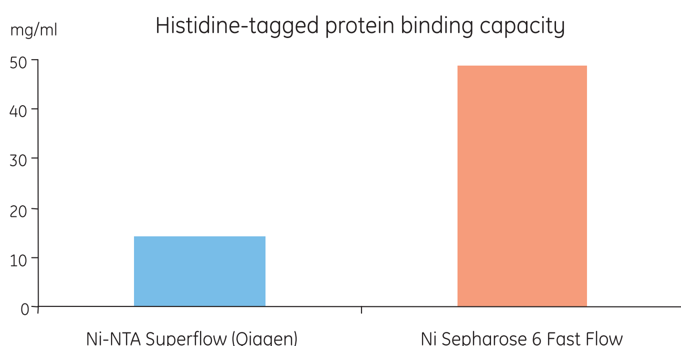


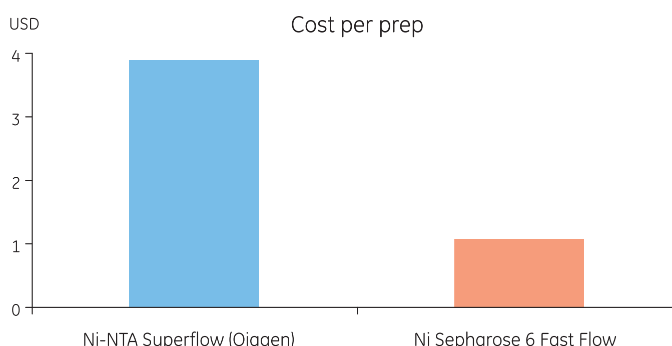
Lane 1: LMW markers
Lane 2: Flow through
Lane 3: Wash 1
Lane 4: Wash 2
Lane 5: Elution fraction 1
Lane 6: Elution fraction 2
Lane 7: Elution fraction 3
Lane 8: Elution fraction 4

(Similar purities were observed also with 0.5 ml of the media.)

Results

- A higher binding capacity results in reduced costs as a consequence of the lower volume requirement of Ni Sepharose 6 Fast Flow.





(Data based on packed bed breakthrough experiments.)

Conclusions

- High binding capacity of Ni Sepharose 6 Fast Flow reduces overall costs of histidine-tagged protein purification.
- Repeated exposure of the medium to reducing agents such as DTT did not affect binding capacity or purity of the histidine-tagged protein.
- Higher yield of batch purified GFP-$(His)_6$ was obtained using Ni Sepharose 6 Fast Flow compared with Ni-NTA Superflow and Ni-NTA Agarose.

Acknowledgement

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Protocols of the comparative studies are found at www.amershambiosciences.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 pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assigne: Hoffman La Roche, Inc).

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