Reliable purification of GST-, His-, and *Strep*-tagged proteins

Working with recombinant affinity-tagged proteins is popular, since it enables easy purification, detection, and immobilization of proteins due to the affinity tag. In addition, protein expression and purification is scalable to the amount required for the downstream application.

Purification of tagged proteins is performed using a bind–wash–elute procedure. Due to the high affinity of the tag to an affinity purification matrix, high-purity proteins (up to 95% pure) are obtained in a single purification step.

The Glutathione S-Transferase (GST) tag, His tag, and *Strep*-tag® II are the three most commonly used purification tags (Table 1). Monoclonal antibodies against all three tags enable specific and sensitive detection of the tagged proteins (Table 2).

Superflow cartridges.

Superflow in bulk format.

Table 1. Overview of technologies

	GST-tag/glutathione technology	His-tag/Ni-NTA technology*	*Strep*-tag II/*Strep*-Tactin® technology†
Tag	26 kDa	6–10 histidine residues with rare influence on protein function, structure, and immunogenicity	8 amino acids with rare influence on protein function, structure, and immunogenicity
Binding capacity of superflow/agarose resin	Up to 20 mg/ml	Up to 50 mg/ml	Up to 9 mg/ml
Special features	Tag increases protein solubility	Purification under denaturing conditions possible (e.g., for purification from inclusion bodies); compatible with a wide range of additives	Well suited for expression from prokaryotic and eukaryotic systems
Automated protocols available	Please inquire	QIAcube®, QIAsymphony® SP, BioRobot®	Please inquire
Maximum pressure of superflow cartridges‡	0.5 MPa; 5 bar		
Recommended flow rate	1 ml cartridge: 0.25–1 ml/min 5 ml cartridge: 1.25–5 ml/min	1 ml cartridge: 1 ml/min 5 ml cartridge: 5 ml/min	1 ml cartridge: 1 ml/min 5 ml cartridge: 5 ml/min

* Available as matrix, magnetic beads, and spin columns.

† Available as matrix and magnetic beads.

‡ Applicable to all common fast protein liquid chromatography (FPLC) instruments.

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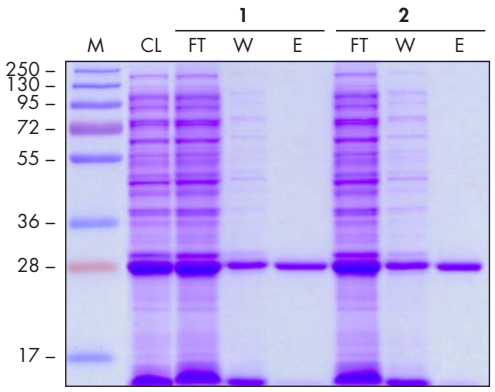

Figure 1. Purification of highly-pure GST-tagged protein using Glutathione HiCap Matrix. GST-tagged protein (19 mg) was purified using Glutathione HiCap Matrix and analyzed by SDS-PAGE. **1–2**: Two purification procedures with same sample source; **CL**: Cleared lysate; **FT** Flow-through; **W**: Wash; **E**: Eluate fractions; **M**: Markers.

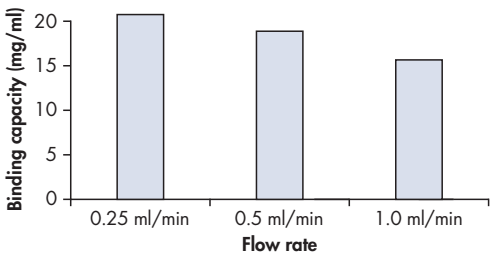

Figure 2. High-capacity dynamic binding of Glutathione HiCap Matrix. Yield of GST-tagged protein achieved using the Glutathione HiCap Cartridge (1 ml) depends on flow rates. Dynamic binding capacity is approx. 20 mg/ml; recommended flow rate for the 1 ml cartridge: 0.25–1 ml/min.

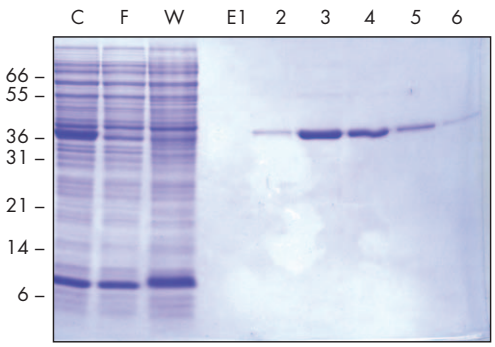

Figure 3. Efficient one-step purification using Ni-NTA Superflow. His-tagged human ERK2 kinase expressed in *E. coli* (100 ml culture volume) was purified using a Ni-NTA Superflow Cartridge by FPLC. ERK2 was eluted with 250 mM imidazole. Yield: 2.5 mg in the eluate. **C**: Cleared lysate; **F**: Flow-through; **W**: Wash; **E1–6**: Elution fractions; **M**: Markers.

Purification of GST-tagged proteins

The GST tag is a versatile tool for purification and detection of proteins. Reliable purification results and increased binding are obtained using Glutathione HiCap Matrix and Cartridges (Figures 1 and 2).

- The GST tag (26 KDa) has a positive influence on protein solubility
- Mild elution conditions maintain protein function
- GST-tagged proteins are highly suitable for pull-down assays
- New Glutathione HiCap resin with up to 50% increased binding

Purification of His-tagged proteins

His-tagged proteins are successfully purified by immobilized metal affinity chromatography (IMAC) using Ni-NTA (Figures 3 and 4).

- Small His tag has negligible influence on protein structure, function, and immunogenicity
- Insoluble proteins purified under denaturing conditions from inclusion bodies
- Purification conditions can be easily adapted to obtain optimal results
- Regulatory support file is available

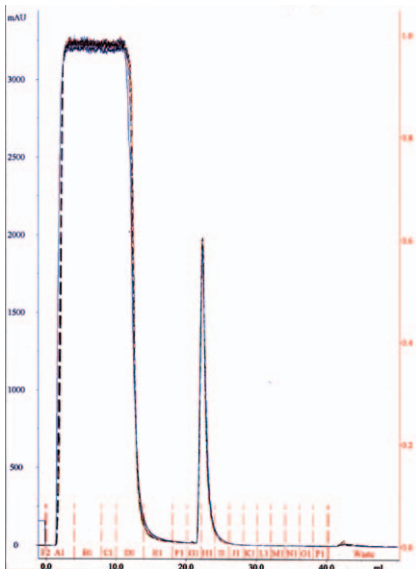

Figure 4. Highly reproducible purification with Ni-NTA. His-tagged pGAPase was purified in 10 sequential purification procedures using FPLC. Column load was 10 ml aliquots of cleared *E. coli* cell lysate containing 30 mg spiked protein. Between purification runs, the column was cleaned in situ using 0.5 M NaOH. Overlay of 10 chromatograms is shown.

Purification of *Strep*-tagged proteins

Highly pure *Strep*-tagged proteins can be obtained using *Strep*-Tactin Superflow Plus (Figure 5). The resin has a 100-fold higher affinity to the *Strep*-tag than streptavidin.

- Highly suitable for purification from prokaryotic and eukaryotic expression systems
- *Strep*-tag II with 8 amino acids (W-S-H-P-Q-F-E-K) has minimal influence on protein structure, function, and immunogenicity
- *Strep*-Tactin Superflow Plus has a 3-fold increased binding capacity (up to 9 mg/ml)
- The *Strep*-tag can be easily combined with the His tag for a two-step purification procedure where ultrapure proteins are required

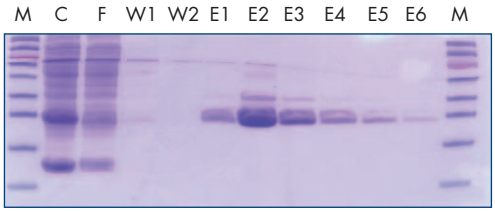

Figure 5. Efficient purification of *Strep*-tagged GFP with *Strep*-Tactin Superflow Plus. GFP was expressed in 60 ml *E. coli* culture and a 5 ml aliquot was applied to *Strep*-Tactin Superflow Plus. Protein was eluted using 2.5 mM desthiobiotin, giving a total protein yield of 3.1 mg. **C**: Cleared lysate; **F**: Flow-through; **W1–2**: Washes; **E1–6**: Elution fractions; **M**: Markers.

Detection of tagged proteins

Mouse monoclonal antibodies without any cross-reactivity to human, mouse, yeast, insect, and *E. coli* proteins allow highly specific detection of tagged proteins (Table 2).

Reliable purification of tagged proteins

QIAGEN's Glutathione, Ni-NTA-, and *Strep*-Tactin technologies provide rapid, reliable purification of high-purity proteins.

Table 2. Antibody specifications

	Penta·His antibody*	GST-tag antibody	*Strep*-tag antibody
Epitope	HHHHH	Glutathione S-transferase from *Schistosoma japonicum*	SAWSHPQFEK
Dissociation constant	5×10^{-8}–1×10^{-9} M	Not determined	5×10^{-9}–1×10^{-10} M
Application	Western blot, dot blot, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunohistochemistry	Western blot, dot/slot blot, ELISA, immunoprecipitation	Western blot, dot blot, ELISA, immunoprecipitation, immunohistochemistry
Sensitivity in western blots (chemiluminescent detection)	50 pg	0.5 ng	1 ng

* Various conjugates available.

Ordering Information

Product	Contents	Cat. no.
Glutathione HiCap Matrix (10 ml)*	20 ml Glutathione HiCap Matrix suspension (50%); for purification of GST-tagged proteins	30900
Glutathione HiCap Cartridges (5 x 5 ml)*	5 cartridges prefilled with 5 ml Glutathione HiCap Matrix; for automated purification of GST-tagged proteins using liquid chromatography systems	30991
Ni-NTA Spin Columns (50)	50 Ni-NTA Spin Columns, Collection Tubes	31014
Ni-NTA Superflow (25 ml)*	25 ml nickel-charged resin†	30410
Ni-NTA Superflow Cartridges (5 x 5 ml)*	5 cartridges prefilled with 5 ml Ni-NTA Superflow; for automated purification of His-tagged proteins using liquid chromatography systems	30761
Ni-NTA Agarose (25 ml)*	25 ml nickel-charged resin (max. pressure: 2.8 psi)	30210
Strep-Tactin Superflow Plus (10 ml)*	10 ml *Strep*-Tactin Superflow Plus resin	30004
Strep-Tactin Superflow Plus Cartridge (5 ml)*	Cartridges prefilled with 5 ml *Strep*-Tactin Superflow; for automated purification of *Strep*-tagged proteins using liquid chromatography systems	30060
GST-tag Antibody (100 µg)	100 µg anti-GST-tag antibody (lyophilized, BSA-free)	34860
Penta·His Antibody, BSA-free (100 µg)‡	100 µg mouse anti-(His)5 (lyophilized, BSA-free; for 1000 ml working solution)	34660
Strep-tag Antibody (100 µg)	100 µg anti-*Strep*-tag II antibody (lyophilized, BSA-free; for 1000 ml working solution)	34850

* Other kit sizes available; see under product name at www.qiagen.com.

† Also available as uncharged NTA resin.

‡ Other antibodies and antibody conjugates available; see www.qiagen.com.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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