nunc™

ProPur™: His-tagged protein purification made easy

ProPur™ affinity spin columns contain a unique flow regulator, FlowGo, which maximizes the contact time between the target protein and the resin. ProPur columns offer the speed of a spin column with the yields and purities of a gravity column or a batch protocol. They are ideal for rapid small-scale and pilot purifications of His-tagged proteins, method development, expression trials, solubility determination and separation of the proteolytically cleaved His tag from the purified protein.

One of the most widely used methods for protein purification is immobilized metal ion affinity chromatography (IMAC), which was introduced in 1975 (ref. 1) and allows rapid one-step purification of fusion proteins. For such procedures, proteins are engineered with affinity tags attached to the 5′ or 3′ end of the target gene. Examples of such tags are hexahistidine and an 8-residue peptide containing alternating histidines. The matrix is attached to chelating groups that immobilize transition metal ions such as Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} (refs. 2–4). Ni^{2+} is the most widely used metal ion as most IMAC tags seem to have very high affinity for immobilized Ni^{2+}. The simplicity of IMAC technology is extremely attractive as it lends itself to the bind-wash-elute mode of operation if the appropriate buffer is selected.

Driven by applications

Nunc's rapid protein purification kit products emerged from a clear and comprehensive understanding of the present methods used by researchers to purify recombinant proteins from prokaryotic and eukaryotic expression systems. With ProPur, the researcher needs a minimum level of experience in protein chromatography to purify their target proteins.

The ProPur metal chelate Mini and Midi kits are designed for simple, complete and rapid purification of His-tagged recombinant protein from bacterial cells, insect vectors, mammalian cells and yeast under native or denaturing conditions (**Table 1**).

Innovation

Our research determined that the vast majority of researchers purifying recombinant proteins work repeatedly with agarose resin. We, therefore, use highly compressible, high-quality, robust and reproducible agarose resin in the Nunc spin columns. We selected the spin-column format because it permits multiple purifications to be performed in parallel and with high reproducibility (for example, low coefficients of variation).

Table 1 | Specifications of the ProPur MC Mini and Midi spin columns

Supporting Nunc ProPur matrix	Covalently coupled to agarose resin
Charged metal ion	Ni^{2+}
Maximum sample volume per load	0.65 ml (Mini, fixed angle rotor) 20 ml (Midi, swing bucket rotor)
Resin bed volume	0.23 ml (Mini) 1.6 ml (Midi)
Bead size range	45–165 µm
Recommended working pH	pH 2–12
Typical number of uses per plug	2 (assuming no further Ni^{2+} charging)
Typical binding capacity per bind-wash-elute cycle	1 mg His-tagged protein (Mini) 20 mg His-tagged protein (Midi)
Chemical stability	High
Plastic construction	Polypropylene
Color-coded end caps	Black

The rapid Mini spin columns can be used to purify up to 24 proteins in a microcentrifuge simultaneously. The ProPur Midi spin columns can be used to purify up to 16 proteins in under 50 min using a standard bench centrifuge.

By precisely controlling how the resin cartridges are packed, we are able to standardize the performance of the cartridge. The column cartridge incorporates a technologically advanced flow regulator, FlowGo, which is designed to control the flow rate of the samples through the active column matrix. Observed yields and purities fluctuate as a direct function of the flow rate of the sample-loading step. The FlowGo is preset by Nunc to slow down the flow rate to an optimal capture speed.

The concomitant increase in the residence time of the target protein with the matrix of the spin column increases substantially the yield and purity of the isolated target protein. The FlowGo functions critically in the sample-loading step, which has been deliberately extended to 30 min for the Midi spin columns and 6 min for the Mini spin columns. Unlike with many other chromatography systems, there are negligible void volumes, and elution of the His-tagged protein to recover the purified target protein is rapid for both Mini (1 min) and Midi (3 min) columns. The patented flow regulator and the different column sizes ensure that researchers can obtain a fairly wide dynamic range of binding capacities.

Mike Brownleader

Thermo Fisher Scientific, Kamstrupvej 90, P. O. Box 90, DK-4000 Roskilde, Denmark. Correspondence should be addressed to V.R. (vibeke.rowell@thermofisher.com).

APPLICATION NOTES

Table 2 | Features and benefits of the ProPur MC spin columns and kits

Benefits	Features
Centrifugal spin column format	Simple to use; no HPLC or FPLC equipment needed; every laboratory has a centrifuge
Uses centrifugal *g* force as driving force	Extremely fast; short separation; no hold-up volume or spluttering; high recovery during fast elution step
Many samples can be centrifuged in parallel	Multiple samples can be purified at the same time
Economical	Low-cost purification
Binding capacity is up to tens of milligrams	Variable volume and amounts can be loaded and recovered with ease
No need for extensive equilibration	Fast pre-equilibration step
Negligible void volume	No loss of critical and precious samples
No need to de-gas the resin slurry	Saves time and mess
Can purify and concentrate the sample in a single step	Saves time as there are fewer steps
Complete elution of proteins	No carryover of proteins or contaminants from previous step
Stable for up to 2 years	No need to store in bacteriocide

The right mix

The ProPur spin columns are precharged with nickel. The kits contain clear and unambiguous protocols, all required buffers and desalting/buffer-exchange columns for purification of His-tagged proteins in less than 50 min using the Midi spin columns and less than 15 min using the Mini spin columns. These metal chelate kits offer a combination of unique features and benefits such as high binding capacity and high selectivity in a Mini and Midi spin column format for less than $1 per milligram of engineered recombinant protein (**Table 2**). They are very simple to use and are ideal for inexperienced chromatography researchers. The spin columns allow highly specific binding and rapid protein purification with often >90–95% purity. The kits can be used to isolate pure target protein from a large amount of contaminants and concentrate the target protein simultaneously.

Modus operandi

Cells lysates are prepared in a typical manner for metal-chelate chromatography. The spin column is pre-equilibrated with binding buffer by centrifuging the column at 500*g* for 3 min. The lysate is filtered through a 0.2-µm filter, and the cleared cell lysate is loaded on to the spin column at an optimal flow rate to ensure efficient capture of the target protein on the nickel chelate matrix. The spin column is then washed to remove unbound proteins, and the purified His-tagged protein is eluted by centrifuging the column with an elution buffer containing the competitor ligand imidazole.

When a recombinant protein is expressed at high levels in *Escherichia coli*, the protein elutes as insoluble aggregates called inclusion bodies. Denaturants such as 6–8 M urea or 6 M guanidinium HCl completely unfold the target protein making the His tag much more accessible for interaction with the matrix. For purifications under denaturing conditions, proteins are eluted with buffer containing imidazole in the presence of a denaturant or with elution buffer with pH from 7.4 to 4.5. The ProPur MC spin kits contain protocols for purification of His-tagged protein under both native and denaturing conditions (**Fig.1**).

The ProPur kits provide high-purity recombinant proteins in a single step. The unique prepacked metal-chelate plugs are simply inserted into the spin columns and placed in a centrifuge. There is no mess, no filling columns, no attachment of accessories, no pumps and no lengthy equilibrations. The spin columns are ideal rapid-purification tools when there are a lot of conditions to test: for example, different expression systems, different constructs, different induction conditions and different purification regimes. Multiple spin columns can be run in parallel to allow purification of larger volumes or different samples before scale-up. The ProPur MC kits permit fast recovery and high yields of pure recombinant proteins.

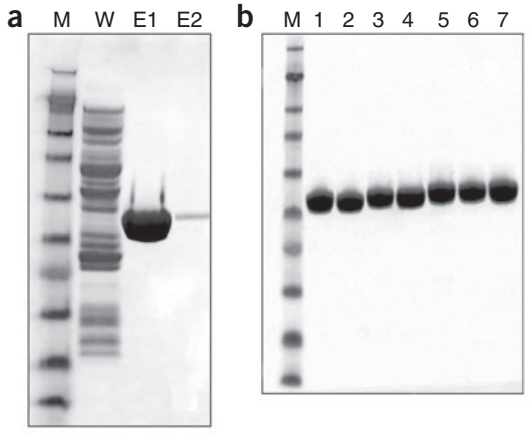

Figure 1 | Purification of His-tagged wild-type and mutant recombinant proteins from *E. coli*. (**a**) Purification of the wild-type protein. M, molecular weight markers; W, sample wash; E1 and E2 are first and second eluates. (**b**) Purification of site-specific mutant His-tagged proteins. M, molecular weight markers; lane 1, purified wild-type His-tagged protein; lanes 2–7, six purified mutant His-tagged proteins.

1. Porath, J., Carlson, J., Olsson, I. & Belfrage, G. Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature* **258**, 598–599 (1975).
2. Porath, J. & Olin, B. Immobilized metal ion affinity adsorption and immobilized metal ion affinity chromatography of biomaterials. Serum protein affinities for gel-immobilized iron and nickel ions. *Biochemistry* **22**, 1621–1630 (1983).
3. Porath, J. IMAC-immobilized metal ion affinity–based chromatography. *Trends Anal. Chem.* **7**, 254–259 (1988).
4. Sulkowski, E. The saga of IMAC and MIT. *Bioessays* **10**, 170 (1989).