Purifying tagged proteins using ÄKTA go protein purification system

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Purifying tagged proteins using ÄKTA™ go protein purification system

What is tagged protein purification?

Tagged protein purification uses affinity chromatography (AC) to purify recombinant proteins that have been engineered to include a specific peptide or protein sequence (tag).

The use of tags significantly simplifies purification and enables use of standard protocols.

How does it work?

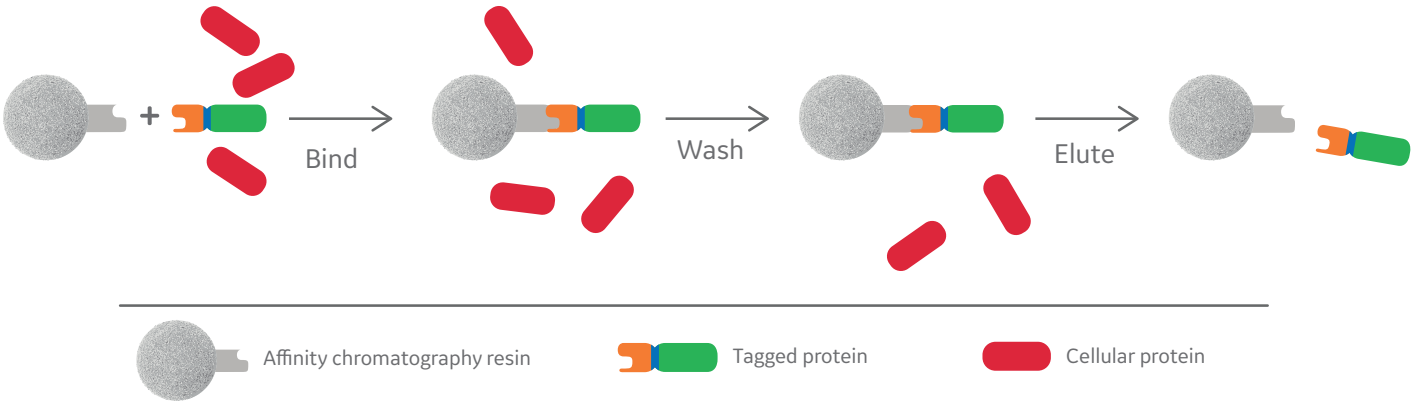
The target protein, with an affinity tag attached, is specifically and reversibly bound to a chromatographic resin containing a binding substance (ligand) with affinity for the tag.

When should tagged protein purification be used?

Affinity purification of tagged proteins can be used as the only purification step in applications that do not require very high purity. When very high purity is needed (95% to 99%), the technique can be used as the first (capture) step followed by a size exclusion chromatography (SEC) step.

Tagged protein purification step-by-step

1. The sample is applied to the column under conditions that favor binding to the ligand. Unbound material is washed out of the column.
2. The bound tagged protein is recovered (eluted), typically using a competitive ligand.
3. The eluted protein is usually at a high concentration. If tag removal is needed prior to use of the protein, cleavage may be performed using a site-specific protease.



Common applications

Tagged proteins are expressed in hosts such as *E. coli*, yeast, as well as insect and mammalian cells. Common choices for protein affinity tags are polyhistidine (histidine-tag), glutathione S-transferase (GST), maltose-binding protein (MBP), *Strep*-tag™ II, and FLAG™ tags. His-tagged proteins are purified with a variant of affinity chromatography called IMAC (immobilized metal affinity chromatography).

Example: his-tagged protein purification using ÄKTA go

ÄKTA go is a small and compact liquid chromatography system that allows researchers to perform routine protein purification with ease while allowing for efficient use of bench and cold cabinet space (Fig 1).

Fig 1. Preparing ÄKTA go for a purification.

His-tagged green fluorescent protein (GFP-His) was purified from an *E. coli* cell extract using two chromatography steps. The columns for each step were connected to the system at the same time using the column valve (Fig 2).

In the first step, affinity purification using a HisTrap™ HP column, a HiTrap™ column prefilled with Ni Sepharose™ HP resin, was used. The eluate was passed on to a second chromatography step using a HiLoad™ 16/600 Superdex™ 75 pg SEC column to further improve purity. Other SEC columns such as HiPrep™ Sephacryl™ S-200 HR can be used depending on, for example, the purity requirements.

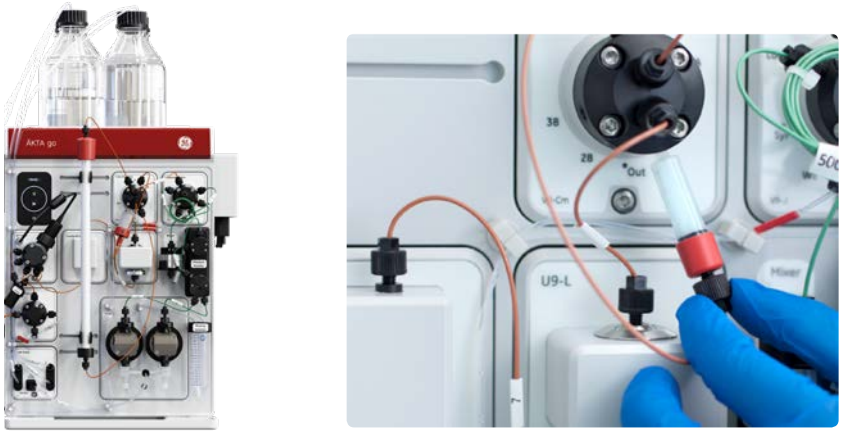
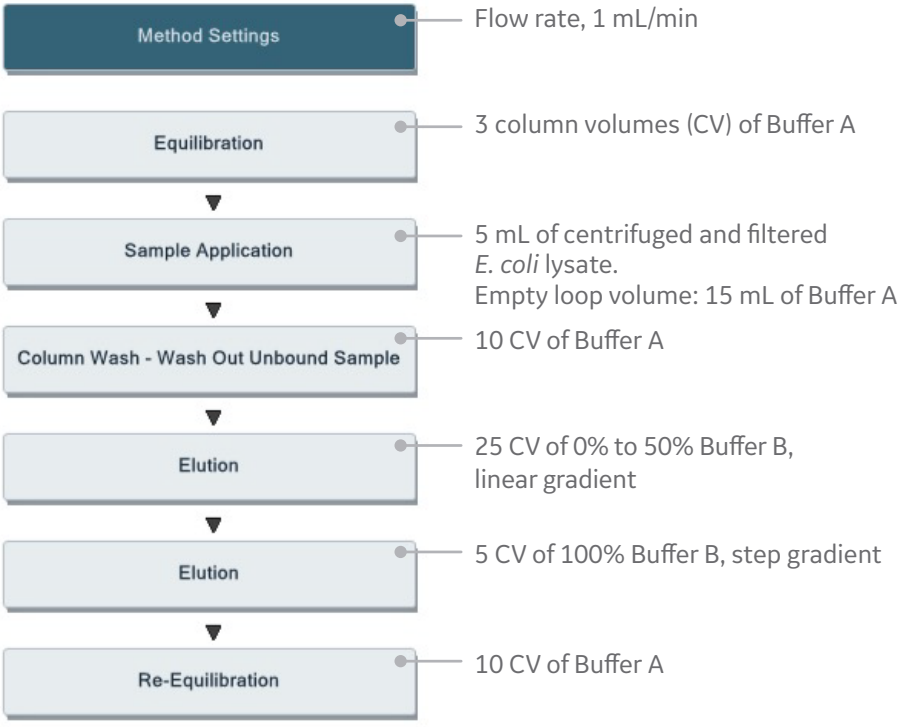


Fig 2. The HisTrap and HiLoad columns were mounted at the same time on ÄKTA go (left) using the optional column valve (right).

Predefined methods simplified the purification

ÄKTA go is fully supported by UNICORN™ software, which gives real-time control of the chromatography system. Automated methods can be created in minutes for most common chromatography techniques using predefined methods. In this application, the methods below were generated using predefined methods (Fig 3).

Affinity chromatography: HisTrap HP, 1 mL



Size exclusion chromatography: HiLoad 16/600 Superdex 75 pg

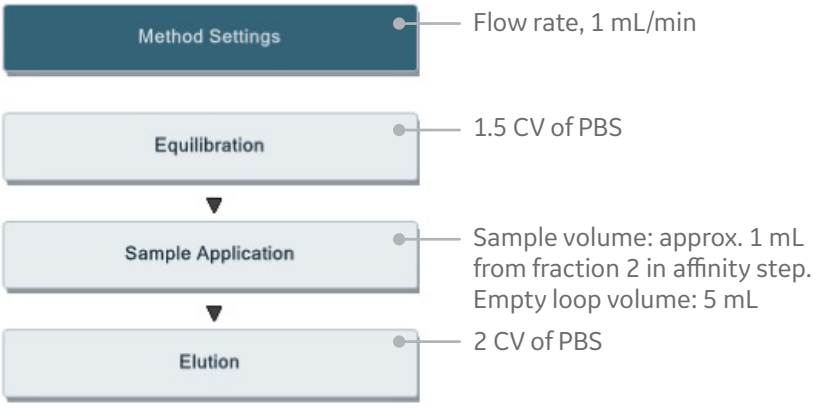


Fig 3. Predefined methods for affinity chromatography and size exclusion chromatography steps in UNICORN software.

Results: efficient and reliable his-tagged protein purification

His-tagged protein purification simplified protein purification and enabled the use of standard protocols. In Figures 4 and 5 results from the two-step protocol are shown. The results from SDS-PAGE verified that GFP-His was effectively purified already after the affinity chromatography step even though some smaller impurities were removed after the final SEC step (Fig 6).

Sample:	GFP-His expressed in *E. coli* cells
Sample prep:	Frozen paste was resuspended, sonicated, and centrifuged; 10 mL of the supernatant was filtered through a 0.45 µm filter
Affinity chromatography:	HisTrap HP, 1 mL (Buffer A: 20 mM sodium phosphate, 500 mM NaCl, pH 7.4; Buffer B: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4)
Size exclusion chromatography:	HiLoad 16/600 Superdex 75 pg (Buffer A: PBS)
Analysis:	SDS-PAGE

Affinity chromatography step: HisTrap HP 1 mL

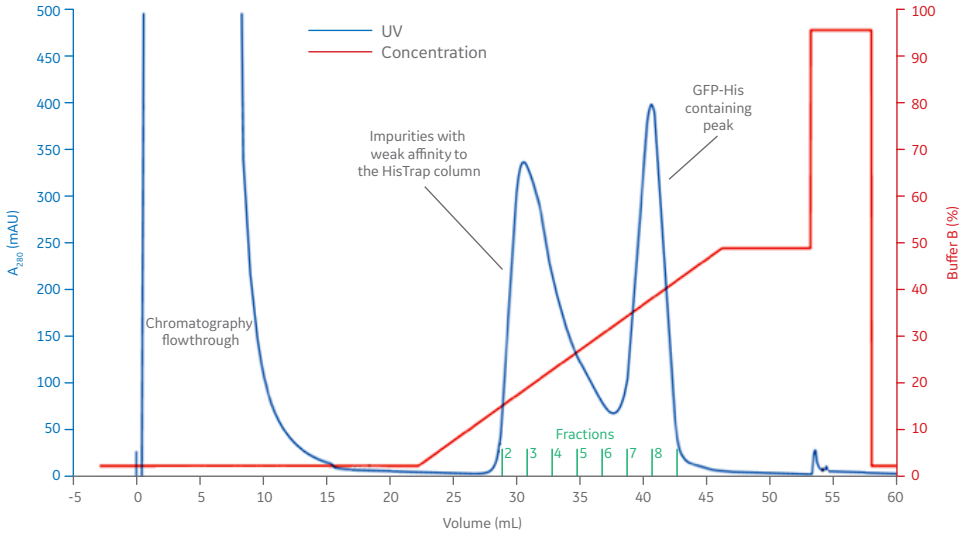


Fig 4. The 5 mL sample of *E. coli* lysate containing GFP-His was applied on a HisTrap HP column. Bound material was eluted by a linear gradient of up to 50% of Buffer B containing 20 mM sodium phosphate, 500 mM NaCl, and 500 mM imidazole. Impurities with weaker affinity to the resin eluted at lower imidazole concentration compared to GFP-His. Fraction 7 and 8, containing GFP-His, were pooled and applied on a HiLoad 16/600 Superdex 75 pg SEC column.

Size exclusion chromatography step: HiLoad 16/600 Superdex 75 pg

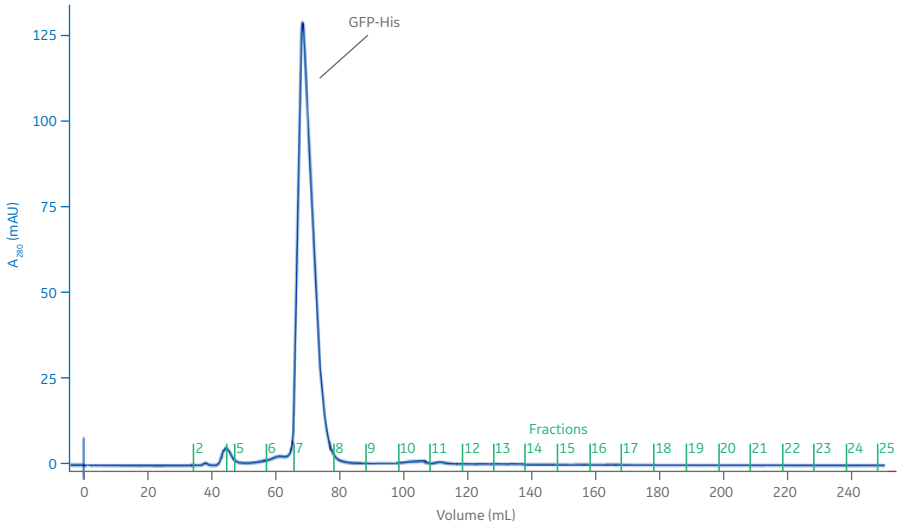


Fig 5. Fractions 7 and 8 from the AC step were pooled to make a total volume of 3.8 mL. The pooled fraction was applied on the HiLoad 16/600 Sephadex 75 pg SEC column.

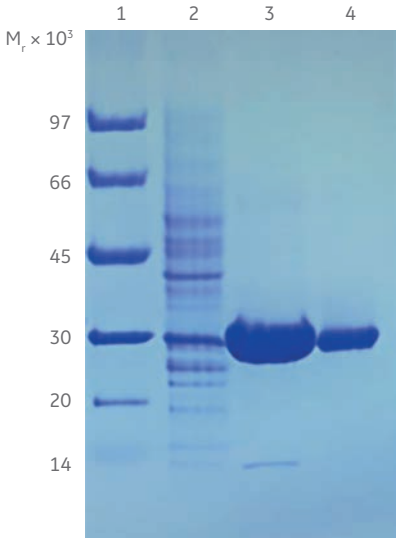


Fig 6. Coomassie™ blue stained SDS-PAGE gel showing GFP-His purity after the two chromatographic steps. Lane 4 shows the purified GFP-His.

Tips for tagged protein purification

- Define the required level of purity and identify options to achieve this level of purity.
- If the sample is not pure enough after SEC, for example, several bands are observed in the SDS-PAGE gel, optimize the AC step or add an extra intermediate purification step such as ion exchange chromatography.
- Determine whether or not you need to remove the tag and how.
- Select the appropriate chromatography resin, format, and instrument that meets your needs.

Resources for more information

- Blog: Simplify every step of histidine-tagged protein purification
- Blog: 5 beginner tips for getting his-tagged protein that meets your needs
- Affinity chromatography handbook Vol.2: Tagged proteins
- How to combine chromatography techniques to optimize your protein purification protocol

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