



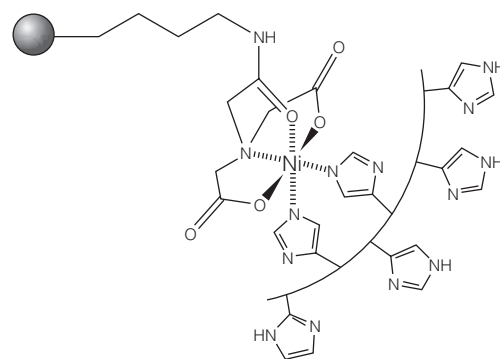
The Strongest Link in the Protein Purification Chain **Promega**

Introducing the HisLink™ Protein Purification Resin

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Abstract

The HisLink™ Protein Purification Resin is a flexible and easy-to-use protein purification resin. The resin uses a modified silica support that increases its ability to capture His-tagged fusion proteins while eliminating nonspecific binding. The HisLink™ Resin can be adapted to many protein purification techniques including column chromatography, batch chromatography and automated methods such as FPLC.



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Figure 1. Schematic of HisLink™ Resin and His-tag interaction. Two sites are available for His-tag binding and are rapidly coordinated with histidine in the presence of a His-tagged polypeptide.

The HisLink™ Protein Purification Resin is user-friendly and adaptable to different protein purification techniques.

Introduction

Expression and affinity-tagged purification of proteins is one of the primary means of obtaining large quantities of recombinant proteins in a purified form. Commonly used affinity tags include poly-histidine (His-tag), glutathione-s-transferase (GST), maltose binding protein and calmodulin. Among these tags, His-tag is the most widely used and has several advantages including: 1) It's small in size thus less immunogenically active, and often it does not need to be removed from the purified protein for downstream applications; 2) The availability of a large number of commercial vectors for expressing His-tagged proteins; 3) The tag may be placed at either the N or C terminus; 4) The interaction of the His-tag does not depend on the tag structure making it possible to purify otherwise insoluble proteins using denaturing conditions.

The affinity interaction that serves as the basis for His-tag purification is believed to be a result of the coordination of a nitrogen on the imidazole moiety of poly-histidine with a vacant coordination site on the metal (Figure 1). The metal, in most cases nickel, is immobilized to a support through complex formation with a chelate that is covalently attached to the support.

The two most common support materials are agarose and silica gel. As a chromatographic support, silica is advantageous because it has a rigid mechanical structure that is not vulnerable to swelling and can withstand large changes in pressure and flow rate without disintegrating or deforming. Silica is available in a wide range of pore and particle sizes including macroporous silica, providing a higher capacity for large biomolecules such as proteins. However, one of the drawbacks of silica as a solid support for affinity purification is the limited reagent chemistry that is available and the relatively low efficiency of surface modification that is obtained.

To overcome these limitations we have developed a new modification process for silica surfaces that provides a tetradentate metal-chelated solid support that has a high binding capacity and concomitantly eliminates the nonspecific binding that is characteristic of unmodified silica. We chose a resin with a pore size that results in binding capacities as high as 35mg of His-tagged protein from one milliliter of resin. Here we introduce the HisLink™ Protein Purification Resin^(a) and demonstrate its versatility for purifying milligram quantities of His-tagged proteins in a variety of fast and convenient formats.

Physical Properties of HisLink™ Resin

The HisLink™ Resin is an ultrapure spherical silica gel with 1,000Å pore size. It has been modified with a proprietary chelating ligand that forms a tetradentate complex with nickel ion (1) at an excess of 20mmol per milliliter of resin and eliminates the nonspecific surface interaction typically found on silica gel. Figure 2 shows the binding efficiency of this resin. When titrated with increasing amounts of a 61kDa purified His-tagged protein, the level of binding at saturation, approximately 100µg/mg of resin, suggests a theoretical capacity of greater than 40mg/ml for this protein.

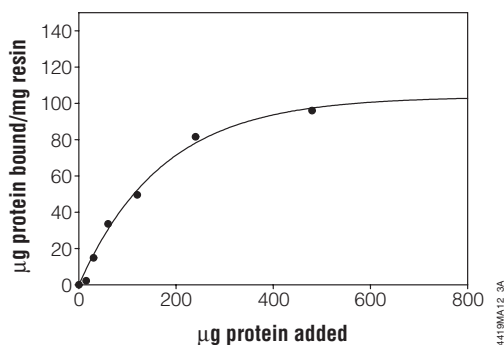


Figure 2. Binding capacity of HisLink™ Resin for a 61kDa His-tagged protein. Purified protein was added in increasing amounts to 1mg of HisLink™ Resin. The amount of protein that remained in the flowthrough fraction was determined by Bradford assay. The difference between the protein measured in the flowthrough fraction and that loaded onto the column was defined as the amount of bound protein. The saturating capacity of 100µg/mg of resin equates to greater than 40mg of protein per milliliter of resin.

Table 1 illustrates the low level of nonspecific interaction, which results from the surface modification. Using a Bradford assay, we measured the amount of non-His-tagged protein recovered from the flowthrough fraction, three wash fractions and the final elution using the lysate of a nontransformed *E. coli* culture (*E. Coli* S30 lysate). Because the lysate used was from a nontransformed culture, any protein that appears in the elution would be considered background in a typical purification. The low level of nonspecific protein in the elution fraction, <1% of the amount loaded, supports the premise that efficient surface modification prevents nonspecific interactions associated with active silica surfaces.

Purification of His-Tagged Proteins

The purification of His-tagged proteins with metal resins follows a simple protocol: bind the His-tagged protein from a cell lysate, wash the resin to remove nonspecific proteins and elute the protein of interest. The procedure is fast and efficient, making it an attractive method of obtaining recombinant protein. The HisLink™ Resin works in a variety of formats typically used to purify His-tagged proteins from lysate. These include batch methods and column purifications driven by gravity or vacuum, or by pressure-applied methods such as Fast

Table 1. Nonspecific binding of *E. coli* protein to HisLink™ Resin. A 100µl aliquot of *E. coli* S30 lysate was loaded onto 1mg of HisLink™ Resin. The lysate was incubated with the HisLink™ Resin, the resin was washed and elution was performed using normal conditions. Aliquots taken from the flowthrough fraction, 3 wash fractions and the elution fraction were quantitated as a percentage of the protein loaded (load set to 100%) by Bradford assay. The percent of eluted protein relative to the lysate measured in the elution was 0.3% in this experiment.

Fraction	% of Total Protein (Adjusted)
Flowthrough	92
Wash 1	5.2
Wash 2	1.6
Wash 3	0.3
Elute	0.3
Total	99.4

Performance Liquid Chromatography (FPLC). User-friendly protocols for each of these different techniques provide similar recovery and purity results with the proteins tested (2). This versatility allows the user to choose the purification method best suited for the equipment and time available for purification.

Buffers Compatible with HisLink™ Resin

Since the binding of a His-tag protein to nickel involves a nitrogen on the imidazole ring of histidine, the pH of the binding and washing steps should be kept in a range that is comfortably above the pH (~6.0) of the imidazole side chain. Suitable buffers include HEPES, Tris and sodium phosphate (Table 2). Salt in the form of sodium chloride is often used to control nonspecific interactions, which may lead to decreased purity of the eluted proteins. Imidazole at a low concentration is also found in most binding buffers to reduce interactions that may be nonspecific in nature. However, the imidazole concentration must be carefully controlled so that binding of the His-tag to nickel is not inhibited.

Table 2. Recommended Buffer Systems for the HisLink™ Protein Purification Resin.

HEPES	
Binding:	100mM HEPES, 10mM imidazole (pH 7.5)
Wash:	100mM HEPES, 10–100mM imidazole (pH 7.5)
Elution:	100mM HEPES, 250–1000mM imidazole (pH 7.5)
Tris	
Binding/Wash:	100mM Tris, 10mM imidazole (pH 7.5)
Elution:	100mM Tris, 500mM imidazole (pH 7.5)
Sodium Phosphate	
Binding/Wash:	50mM sodium phosphate, 300mM NaCl, 10mM imidazole (pH 8.0)
Elution:	50mM sodium phosphate, 300mM NaCl, 250mM imidazole (pH 8.0)

Using imidazole is the most common and efficient mean for eluting bound protein from the resin. Typically His-tagged proteins bound to HisLink™ Resin can withstand imidazole concentrations between 10–100mM without eluting significantly from the resin. Elution is protein dependent, with most eluting in the range of 250–500mM imidazole.

We used a HEPES-based buffer system for work reported here (Table 2). This buffer system offered an excellent compromise between purification performance and stability of the purified protein. When buffers other than HEPES are used, purifications performed in the absence of protease inhibitors produce a significant difference in the amount of degraded protein observed by Coomassie® staining (data not shown). Controlling this degradation is important since potentially undesirable His-tagged protein fragments can co-purify with the full-length protein. HEPES also shows a much lower dependency on the presence of salt to reduce nonspecific interactions, and in most cases adding salt was not required for clean elution of the protein. The compositions of the buffer systems we recommend are shown in Table 2.

Column Purification of His-Tagged Proteins

Column purification with HisLink™ Resin provides a conventional means to purify His-tagged proteins and requires only a column that can be packed to the appropriate bed volume. If packed to 1ml under gravity-driven flow, HisLink™ Resin shows an average flow rate of approximately 1ml/minute. In general a flow rate of 1–2ml/minute per milliliter of resin is optimal for efficient capture of His-tagged protein. The results of column purification of two moderately expressing proteins are shown in Figure 3.

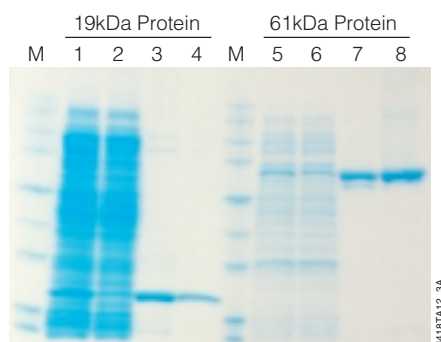


Figure 3. Purification of 19kDa and 61kDa fusion proteins using the HisLink™ Resin. One liter of an IPTG-induced culture of a 19kDa His-tagged protein was spun down and lysed at a 50-fold concentration (20ml of lysate). One liter of an IPTG-induced *E. coli* culture expressing a 61kDa His-tagged protein was concentrated tenfold. After clearing, the proteins were purified on 1ml HisLink™ Resin columns using a binding/wash buffer consisting of 100mM HEPES and 10mM imidazole (pH 7.5) and an elution buffer consisting of 100mM HEPES and 500mM imidazole (pH 7.5). Lanes 1 and 5, bacterial cell lysate expressing 6X His-tagged protein; lane 2 and 6, flowthrough of lysate; lane 3,4,7 and 8, respectively, are consecutive elution fractions of purified proteins. Lanes M, Broad Range Protein Markers (Cat.# V8491).

Batch Purification of His-Tagged Proteins

One of the primary advantages of the HisLink™ Resin is its use in batch purification. In batch mode, the protein of interest is bound to the resin by mixing lysate with the resin for a period of approximately 30 minutes at a temperature range of 4–22°C. Once bound with protein, the resin is allowed to settle to the bottom of the container, and the spent lysate is poured off. Washing only requires resuspension of the resin in an appropriate wash buffer followed by a brief period to allow the resin to settle. The wash buffer is then carefully poured off. This process is repeated as many times as desired. Final elution is best achieved by transferring the HisLink™ Resin to a column for elution of the protein in fractions.

The advantages of batch purification are: 1) Less time is required to perform the purification, 2) Large amounts of lysate can be processed, and 3) Clearing the lysate prior to purification is not required. Figure 4 shows proteins purified with HisLink™ Resin using gravity-flow column and batch methods with and without clearing. The resulting quality and the amount of purified protein was independent of the method used for purification.

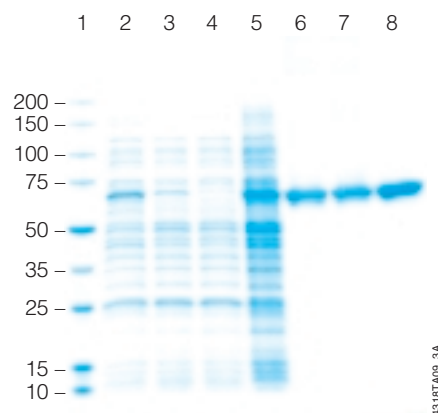


Figure 4. Purification of 6X His-tagged fusion protein using HisLink™ Resin in a column and batch format with both cleared and crude lysate. Lane 1, Broad Range Protein Markers (Cat.# V8491); lane 2, bacterial lysate expressing 6X His-tagged protein; lane 3, flowthrough of cleared lysate (column method); lane 4, flowthrough of cleared lysate (batch); lane 5, flowthrough of crude lysate (batch); lane 6, elution of 6X His-tagged protein from cleared lysate (column); lane 7, elution of 6X His-tagged protein from cleared lysate (batch); lane 8, elution of 6X His-tagged protein from crude lysate (batch). The binding and wash buffers were the same as described for Figure 3.

HisLink™ Protein Purification Resin... continued

Purification by FPLC

The rigid particle structure of the silica base used in the HisLink™ Resin make this material an excellent choice for applications that require applied pressure to load the lysate, wash or elute protein from the resin. These applications include both manual and automated systems that operate under positive or negative pressure (e.g., FPLC and vacuum systems, respectively).

To demonstrate the use of HisLink™ Resin on an automated platform we used an AKTA explorer from Amersham Biosciences. Milligram quantities of his-tagged protein were purified from one liter of culture. The culture was lysed in 20ml of binding/wash buffer and loaded onto a column containing 1ml of HisLink™ Resin. Figure 5 shows the chromatogram produced by monitoring the process at 280nm and the gel analysis of the lysate, flowthrough and elution fractions. We estimate the total amount of protein recovered to be between 75–90% of the protein expressed in the original lysate.

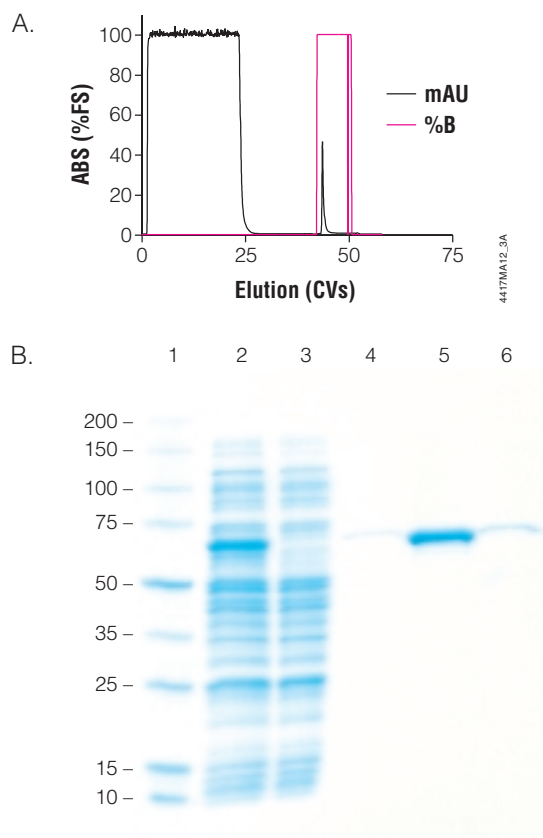


Figure 5. Purification of 6X His-tagged fusion protein using HisLink™ Resin and AKTA explorer (Amersham Biosciences). The system was programmed to load the lysate from a 50ml superloop, wash the column with 20 column-volumes of wash buffer and immediately elute and collect fractions. The binding/wash and elution buffers were the same as described for Figure 3. **Panel A.** Chromatogram monitored at 280nm. Pink line is buffer gradient. **Panel B.** Lane 1, Broad Range Protein Markers (Cat.# V8491); lane 2, bacterial lysate expressing 6X His-tagged protein; lane 3, flowthrough of lysate; lanes 4, 5 and 6, respectively, consecutive elution fractions of the purified protein.

Conclusion

The HisLink™ Protein Purification Resin is a macroporous silica resin modified to contain a high level of tetradentate chelated nickel (> 20mmol/ml of settled resin) for efficient capture and purification of bacterially expressed His-tagged fusion proteins. The resin lacks the nonspecific binding characteristics typically associated with silica. The binding capacity of the HisLink™ Resin is $\geq 15\text{mg/ml}$ ($>1,000\text{nmol/ml}$) for an 8kDa protein. Recoveries as high as 35mg/ml have been observed with a 61kDa protein ($>500\text{nmol/ml}$).

The HisLink™ Resin is user-friendly and adaptable to different protein purification techniques including conventional column chromatography, batch chromatography and automated methods such as FPLC. In batch mode, the HisLink™ Resin has the added advantage of being easily separated from the binding and wash solutions, greatly speeding up the purification process and eliminating the need to clear lysates prior to purification.

Protocol

- ◆ HisLink™ Protein Purification Resin Technical Bulletin #TB327, Promega Corporation. (www.promega.com/tbs/tb327/tb327.html)

References

1. Motekaitis, R.J. and Martell, A.E. (1974) *Inorg. Chem.* **13**, 550–9.
2. HisLink™ Protein Purification Resin Technical Bulletin #TB327, Promega Corporation.



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Ordering Information

Product	Size	Cat.#
HisLink™ Protein Purification Resin ^(a)	50ml	V8821

^(a)Certain applications of this product are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

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