TECHNICAL MANUAL

HisLink™ Spin Protein Purification System

Instructions for Use of Product
V1320

Revised 8/15
TM281

HisLink™ Spin Protein Purification System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The HisLink™ Spin Protein Purification System[a,b,c] provides a simple, quick and robust method for purifying polyhistidine- or HQ-tagged expressed proteins from a 700µl sample of *E. coli* cell culture, using either a centrifuge- or vacuum-based method.

HQ (HisGln) tags developed by Promega perform in a manner similar to polyhistidine tags and are available as N- or C-terminal fusion tags in the HQ-tagged Flexi® Vectors. See Section 6.C for details on these vectors.

Protein can be purified directly from culture medium containing bacterial cells expressing a polyhistidine- or HQ-tagged protein. The bacterial cells are lysed using FastBreak™ Cell Lysis Reagent, followed immediately by addition of HisLink™ Protein Purification Resin to the culture. Addition of these reagents results in simultaneous bacterial lysis and binding of the polyhistidine- or HQ-tagged proteins. The samples are then transferred to a Spin Column where unbound protein is washed away and the target protein is recovered by elution. This system requires the use of a tabletop centrifuge or vacuum manifold (Figure 1). A schematic diagram of protein purification using the HisLink™ Spin System is shown in Figure 2.

Advantages of the HisLink™ Spin Protein Purification System include:

- **Simple:** No cell culture preparation steps.
- **Quick:** No lengthy lysozyme incubations required to lyse cells.
- **Efficient:** Binding capacity of 1mg of polyhistidine-tagged protein per spin column.

Figure 1. The Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231).

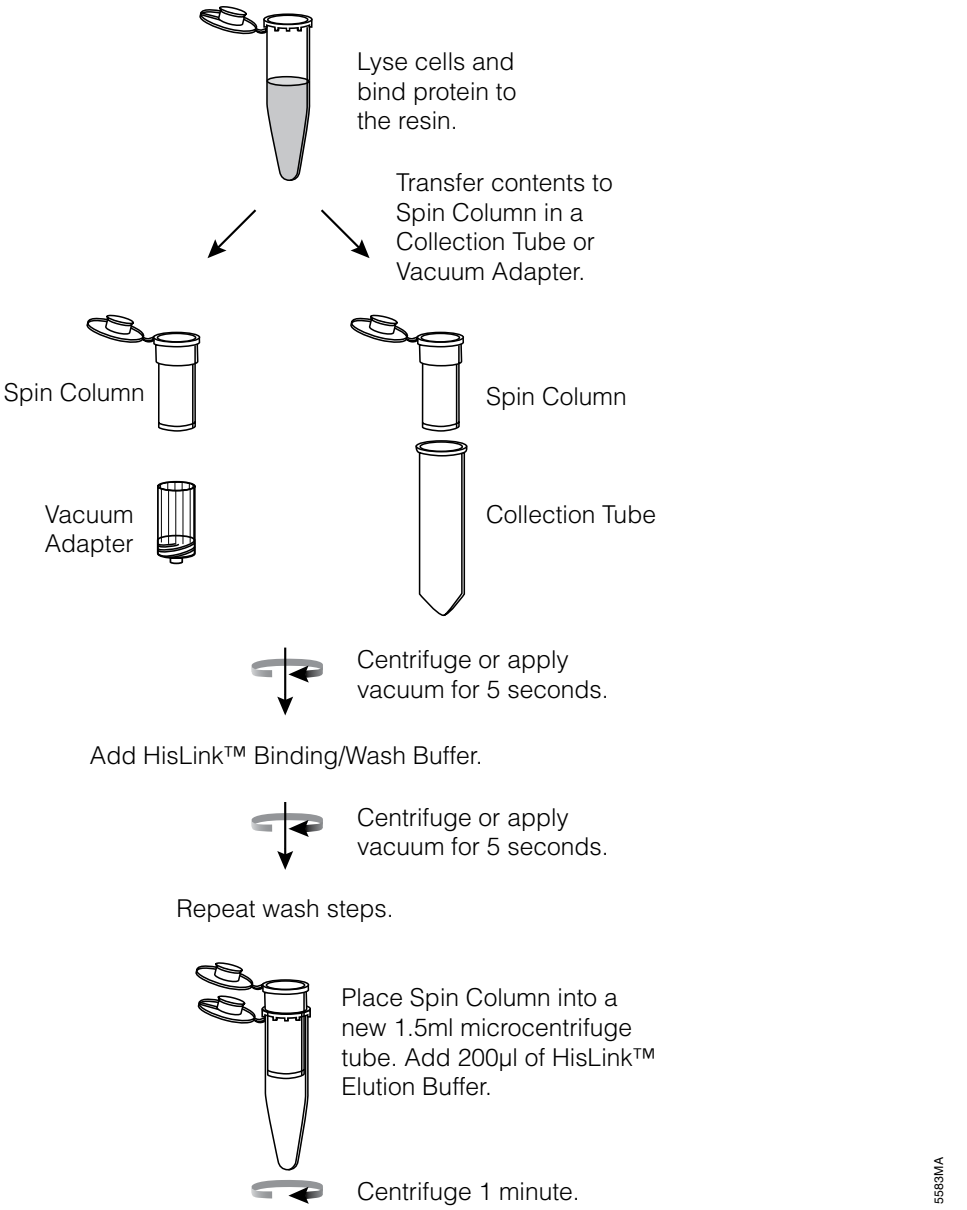

Figure 2. Schematic of polyhistidine- or HQ-tagged protein purification using the HisLink™ Spin Protein Purification System.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
HisLink™ Spin Protein Purification System	**25 reactions**	**V1320**

Each system contains sufficient reagents for 25 manual purifications from 700µl of bacterial culture. Includes:

- 5ml FastBreak™ Cell Lysis Reagent, 10X
- 1 vial DNase I (lyophilized)
- 5ml HisLink™ Protein Purification Resin
- 50ml HisLink™ Binding/Wash Buffer
- 10ml HisLink™ Elution Buffer
- 25 Spin Columns
- 25 Collection Tubes
- 1 Protocol

Storage Conditions: Store the HisLink™ Spin Protein Purification System reagents at 4°C. Store the Spin Columns, Collection Tubes and FastBreak™ Cell Lysis Reagent at room temperature (22–25°C). After reconstitution, store the DNase I in aliquots at –20°C. A precipitate may form in the FastBreak™ Cell Lysis Reagent at low temperatures. If a precipitate forms, warm the reagent to room temperature before use. **Do not freeze** the HisLink™ Resin.

3. Polyhistidine- or HQ-Tagged Protein Purification Protocols

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.A.)

- Nuclease-Free Water (Cat.# P1195)
- rotor
- wide-bore pipette tips (E&K Scientific Cat.# 3502 - R96S)
- NaCl or 5M NaCl solution for use with HQ-tagged proteins
- tabletop centrifuge
- 1.5ml microcentrifuge tubes
- **optional:** vacuum manifold for single tubes if using the vacuum protocol in Section 3.F (i.e., Vac-Man® Laboratory Vacuum Manifold, Cat.# A7231)
- **optional:** Vacuum Adapters (Cat.# A1331; for use with Vac-Man® Vacuum Manifold)

3.A. Preparation of Cell Cultures

Bacterial cultures can be grown in tubes or flasks. Grow the bacterial cells containing a vector encoding the appropriate polyhistidine- or HQ-tagged fusion protein to an $O.D._{600}$ of 0.4–0.6, then induce protein expression. For IPTG induction, add IPTG to a final concentration of 1mM and incubate at 37°C for 3 hours or at 25°C overnight. Induction time and IPTG concentration may require optimization. Cultures with concentrations of up to 8.0 $O.D._{600}$ units/ml have been successfully used with the HisLink™ Spin Protein Purification System. Centrifugation or freezing of cells is not required with this system. A maximum of 700µl of bacterial culture can be loaded per Spin Column.

3.B. Considerations When Adding Lysozyme

The HisLink™ Spin Protein Purification System is designed to lyse cells without the addition of lysozyme. Lysozyme, if added, will copurify with the polyhistidine- or HQ-tagged protein unless 500mM NaCl is added to the Binding/Wash Buffer.

3.C. Considerations When Using HQ-Tagged Proteins

HQ-tagged protein binding is optimized in the presence of NaCl; a maximum of 200mM NaCl final concentration is recommended. Higher NaCl concentrations during binding can cause clumping of the HisLink™ Resin. If NaCl is added to the HisLink™ Binding/Wash Buffer, we recommend its use in both binding and washing steps.

Purification of HQ-tagged proteins may be more efficient from a pelleted culture. Compare direct purification from culture to purification from pelleted cells to verify which is more efficient for the target HQ clone.

To pellet cells, centrifuge at 8,000rpm for 2 minutes, then resuspend in 700µl of 100mM HEPES and follow the standard protocol (Section 3.D, followed by Section 3.E or 3.F). For optimal HQ-tagged protein binding, add a maximum of 200mM NaCl to the 100mM HEPES.

3.D. Preparation of FastBreak™ Reagent/DNase I Solution

1. To prepare the FastBreak™/DNase I solution, add 80µl of Nuclease-Free Water to the vial of DNase I.
2. Mix completely to dissolve the powder.
3. Remove the DNase solution from the vial and add it to 1ml of Nuclease-Free Water. Mix well.

   Note: Once resuspended, the DNase I solution can be stored at –20°C for 6 months and is stable for 8 freeze-thaw cycles.

3.D. Preparation of FastBreak™ Reagent/DNase I Solution (continued)

4. Prepare the FastBreak™ Reagent/DNase I solution using the reagent quantities suggested in Table 1.

   Note: Once prepared, the FastBreak™ Reagent/DNase I solution can be stored at –20°C for 6 months and is stable for up to 5 freeze-thaw cycles.

Table 1. Reagent Volumes for Preparing the FastBreak™ Reagent/DNase I Solution.

# of Reactions	DNase I Solution	FastBreak™ Reagent, 10X
1	5.8µl	64.2µl
2	11.6µl	128.4µl
4	23.2µl	256.8µl
8	46.4µl	513.6µl
16	93µl	1,027µl

3.E. Centrifugation Protocol

1. Pipet 700µl of bacterial culture into a 1.5ml microcentrifuge tube. Add 70µl of the FastBreak™ Reagent/DNase I solution prepared in Section 3.D, Table 1, to the culture.

   Note: For HQ-tagged proteins, please see Section 3.C.

2. Resuspend the resin and allow it to settle. Once the resin has settled, use a wide-bore pipette tip to transfer 75µl of the HisLink™ Resin from the settled resin bed to the 1.5ml microcentrifuge tube. To successfully transfer resin, place the wide-bore pipette tip deep into the resin and pipet slowly to assure that a consistent amount of resin is drawn into the pipette. Allow the resin to resettle between each pipetting.

   Note: We recommend optimizing the amount of HisLink™ Resin used for low- (<1mg/ml) or high- (>1mg/sample) expressing proteins. For low-expressing proteins, less resin should be used; similarly, for high-expressing proteins, more resin per sample can be used.

3. Incubate the sample and resin for 30 minutes, mixing frequently on a rotating platform or shaker to optimize binding.

4. Place a Spin Column onto a Collection Tube (or a new 1.5ml microcentrifuge tube). Use a wide-bore pipette tip to transfer the lysate and resin from the original 1.5ml microcentrifuge tube in Step 3 to the spin column. If resin remains in the 1.5ml microcentrifuge tube, add HisLink™ Binding/Wash Buffer to the tube, then transfer the buffer and remaining resin to the spin column.
5. Centrifuge the spin column with the collection tube for 5 seconds or until the liquid clears the spin column.
6. To save the flowthrough, remove the spin column from the collection tube and transfer the flowthrough from the collection tube to a new 1.5ml microcentrifuge tube. Otherwise, discard the flowthrough.
7. Place the spin column back onto the collection tube. Add 500µl of HisLink™ Binding/Wash Buffer to the spin column, then cap the spin column. Centrifuge for 5 seconds or until the Binding/Wash Buffer clears the spin column. Discard the flowthrough. Repeat for a total of two washes.
8. Take the spin column off the collection tube and wipe the base of the spin column with a clean absorbent paper towel to remove any excess HisLink™ Binding/Wash Buffer.
9. Place the spin column onto a new 1.5ml microcentrifuge tube. Add 200µl of HisLink™ Elution Buffer. Cap the spin column and tap or flick it several times to resuspend the resin. Wait 3 minutes.

   Note: HQ-tagged proteins may elute with a lower concentration of imidazole (50–100mM) compared to polyhistidine-tagged proteins. Section 4.A has details on the use of imidazole for elution.
10. Centrifuge the spin column and microcentrifuge tube at 14,000rpm for 1 minute to collect the eluted protein.

3.F. Vacuum Protocol

1. Pipet 700µl of bacterial culture into the Collection Tube or a 1.5ml microcentrifuge tube. Add 70µl of the FastBreak™ Reagent/DNase I solution prepared in Section 3.D, Table 1, to the bacterial culture.

   Note: For HQ-tagged proteins, please see Section 3.C.

2. Resuspend the resin and allow it to settle. Once the resin has settled, use a wide-bore pipette tip to transfer 75µl of the HisLink™ Resin from the settled resin bed to the tube containing the culture. To successfully transfer resin, place the wide-bore pipette tip deep into the resin and pipet slowly to ensure that a consistent amount of resin is drawn into the pipette. Allow the resin to resettle between each pipetting.

   Note: We recommend optimizing the amount of HisLink™ Resin used for low- (<1mg/ml) or high- (>1mg/sample) expressing proteins. For low-expressing proteins, less resin should be used; similarly, for high-expressing proteins, more resin per sample should be used.

3. Incubate the sample and resin for 30 minutes, mixing frequently on a rotating platform or shaker to optimize binding.

4. Place a spin column onto a Vacuum Adapter (Cat.# A1331), then attach the adapter to a vacuum port. Use a wide-bore pipette tip to transfer the lysate and resin from Step 3 to the spin column. If resin remains in the 1.5ml microcentrifuge tube, add HisLink™ Binding/Wash Buffer then transfer the remaining resin to the spin column.

   ! Any unused ports on the vacuum manifold must be closed for the manifold to work properly.

5. Apply a vacuum for 5 seconds or until the lysate clears the spin column.

6. Add 500µl of HisLink™ Binding/Wash Buffer to the spin column. Apply a vacuum for 5 seconds. Repeat for a total of two washes.

7. Take the spin column off the vacuum adapter and wipe the base of the spin column with a clean absorbent paper towel to remove any excess HisLink™ Binding/Wash Buffer.

8. Place the spin column onto a new 1.5ml microcentrifuge tube. Add 200µl of HisLink™ Elution Buffer. Cap the spin column and tap or flick it several times to resuspend the resin. Wait 3 minutes.

   Note: HQ-tagged proteins may elute with a lower concentration of imidazole (50–100mM) compared to polyhistidine-tagged proteins.

9. Centrifuge the spin column with the 1.5ml microcentrifuge tube at 14,000rpm for 1 minute to collect the eluted protein.

4. General Considerations

4.A. Elution

Imidazole (50–1,000mM): For certain proteins and purification tags, elution efficiency may need to be optimized. Polyhistidine-tagged proteins can be eluted using 250–1,000mM imidazole. Polyhistidine-tagged proteins containing less than six histidines typically require less imidazole for elution, while such proteins containing more than six histidines may require higher levels of imidazole. The HQ tag contains three histidines and three glutamines (HQHQHQ) and may be eluted with a lower concentration of imidazole (50–100mM).

EDTA (>100mM): Strong chelators such as EDTA will strip nickel from the resin and release bound protein. EDTA is generally not as efficient as imidazole for elution, and it has the added complication that Ni(EDTA) will be present in the eluate and must be removed.

Acidic pH: Polyhistidine binding to immobilized nickel is most efficient at a pH well above the pKa of polyhistidine (~ 6.0). Above pH 7, more than 90% of the imidazole moieties of histidine will be deprotonated and available to bind to nickel (1). Lowering the pH of a binding reaction below the pKa of histidine leads to protonation of the polyhistidine tag and release from the resin. Buffers that can be used at this low pH for elution include citrate and acetate (pH 3–5). Trifluoroacetic acid (e.g., 0.1% TFA) can also be used for elution, and because of its volatility, it is directly compatible with mass spectrometry analysis. As with EDTA elution, acidic elution is generally not as efficient as imidazole elution and can result in leaching of nickel, which may become quite significant at pH values below 4. These considerations also apply to HQ-tagged proteins.

4.B. Denaturing Conditions

Proteins that are expressed as inclusion bodies or insoluble fractions should be solubilized with chaotropic agents such as guanidine-HCl or urea before purification. Solubilized proteins can be purified by modifying the above protocols to include the appropriate amount of denaturant (up to 6M guanidine-HCl or up to 8M urea) during binding.

4.C. Reuse of the Resin

Due to the drying of the particles during the elution step, we do not recommend reuse of the particles.

4.D. Adjuncts for Lysis or Purification

We have found that the following materials may be used without adversely affecting the ability of HisLink™ Protein Purification Resin to bind and elute polyhistidine- or HQ-tagged proteins.

Table 2. Reagents Compatible with the HisLink™ Spin Protein Purification System.

Additive	Concentration
HEPES, Tris or sodium phosphate buffers	≤100mM
Triton® X-100	≤2%
Tween®	≤2%
glycerol	≤20%
guanidine-HCl	≤6M
urea	≤8M
RQ1 RNase-Free DNase	≤5µl/1ml original culture

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Possible Causes and Comments
Low amounts of or no protein recovered	Protein expression not induced. Check concentration and stability of inducer (e.g., IPTG).
	Expressed protein may not be stable. Add protease inhibitors to the lysis step. If the protein is degraded at the time of expression, reduce the induction period. Also try using a lower temperature during induction (16–20°C).
	Protein may be expressed in low amounts. Try different temperatures during induction. Check by Western blotting if using polyhistidine tag. Confirm that the sequence of the clone is correct.
	Protein may be misfolded or expressed in inclusion bodies. Check the lysate, pellet or flowthrough for the presence of insoluble protein of interest.

5. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
Low amounts of or no protein recovered (continued)	Cells not lysing properly. Be sure to use the recommended volume of FastBreak™ Cell Lysis Reagent, 10X.
	Particles and lysate are not well mixed. Mix frequently for 30 minutes. Protein may have metal binding domain. Try eluting with a higher concentration of imidazole or with acidic conditions such as citrate.
	Polyhistidine- or HQ-tagged protein may purify more efficiently from pelleted cells. Centrifuge cells 2 minutes at 8,000rpm, discard supernatant and resuspend pellet in 700µl of 100mM HEPES, then follow protocol in Sections 3.D and 3.E or 3.F. To optimize HQ-tagged protein binding, add a maximum of 200mM NaCl to the 100mM HEPES.
Co-elution of contaminating proteins	There may be an interacting protein in the cell. Add 0.5–1M NaCl (final concentration) to the Binding/Wash Buffer.
	Add protease inhibitors to the FastBreak™ Reagent/DNase I solution, Binding/Wash and Elution Buffers to prevent possible protein degradation.
Lysate not clearing the Spin Column	Incorrect amount of DNase I may have been added. Check that the DNase I was diluted properly (Section 3.D).
	DNase I no longer active. Try using a new aliquot of DNase I. Store resuspended DNase I in aliquots at –20°C.
	Culture $O.D._{600}$ exceeds system recommendations (≤8 $O.D._{600}$). Adjust the cell concentration or add more DNase I.
	Unused vacuum manifold ports were not closed. Close all unused ports to ensure vacuum efficiency.
Wash solution not clearing	Poor vacuum efficiency. Check vacuum connection to the manifold to be sure that the hoses are connected properly.
	Unused manifold ports were not closed. Seal all unused ports to ensure good vacuum efficiency.
	Resin clumping due to inactive DNase I or high salt content. Pipet to break up clumps, then repeat wash steps.

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6. Appendix

6.A. Composition of Buffers and Solutions

HisLink™ Binding/Wash Buffer (pH 7.5)

100mM HEPES
10mM imidazole

HisLink™ Elution Buffer (pH 7.5)

100mM HEPES
500mM imidazole

6.B. Reference

1. Hochuli, E. (1990) Purification of recombinant proteins with metal chelate adsorbent. *Genet. Engineer. News* **12**, 87–98.

6.C. Related Products

Product	Size	Cat. #
Vac-Man® Laboratory Vacuum Manifold	1 each	A7231
HisLink™ 96 Protein Purification System	1 × 96	V3680
	5 × 96	V3681
HisLink™ Protein Purification Resin	50ml	V8821
	5ml	V8823
MagneHis™ Protein Purification System	65 reactions	V8500
	325 reactions	V8550
MagZ™ Protein Purification System	30 purifications	V8830
MagneGST™ Protein Purification System	40 reactions	V8600
	200 reactions	V8603
Broad Range Protein Molecular Weight Markers	100 lanes	V8491
Gel Drying Kit, 17.5 × 20 cm capacity	1 kit	V7120

HQ Tag Flexi® Vectors

Product	Size	Cat.#
pFN6A (HQ) Flexi® Vector	20µg	C8511
pFN6K (HQ) Flexi® Vector	20µg	C8521
pFC7A (HQ) Flexi® Vector	20µg	C8531
pFC7K (HQ) Flexi® Vector	20µg	C8541

pFN6A and pFN6K (HQ) Flexi® Vectors: These vectors are designed for expressing N-terminal, HisGln (HQ) metal-binding peptide fusion proteins in bacteria and in vitro protein expression systems. The vectors are configured to append the peptide sequence MKHQHQHQAIA to the amino terminus of a protein. The vectors are designed for bacterial or in vitro protein expression via the T7 RNA polymerase promoter and are available with ampicillin [pFN6A (HQ) Flexi® Vector] or kanamycin [pFN6K (HQ) Flexi® Vector] resistance for selection in *E. coli*.

pFC7A and pFC7K (HQ) Flexi® Vectors: These vectors are designed for expressing C-terminal, HisGln (HQ) metal-binding peptide fusion proteins in bacteria and in vitro protein expression systems. The vectors are configured to append the peptide sequence VSHQHQHQ to the carboxy terminus of a protein. The vectors are designed for bacterial or in vitro protein expression via the T7 RNA polymerase promoter and are available with ampicillin [pFC7A (HQ) Flexi® Vector] or kanamycin [pFC7K (HQ) Flexi® Vector] resistance for selection in *E. coli*.

For further information regarding these HQ Flexi® Vectors and the Flexi® Cloning Systems, refer to the *Flexi® Vector System Technical Manual* #TM254 or visit: **www.promega.com**

7. Summary of Changes

The following change was made to the 8/15 revision of this document:

1. The document design was updated.

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