



Promega

Technical Bulletin

HisLink™ 96 Protein Purification System

INSTRUCTIONS FOR USE OF PRODUCTS V3680 AND V3681.



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HisLink™ 96 Protein Purification System

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1. Description

The HisLink™ 96 Protein Purification System^(a,b,c) provides a simple, quick and robust method of purifying multiple polyhistidine- or HQ-tagged expressed proteins from *E. coli* using a vacuum-based method. The system is designed to purify expressed polyhistidine- or HQ-tagged proteins directly from deep-well, 96-well plates. The HisLink™ System is amenable to manual or automated methods, such as the Beckman Coulter Biomek® 2000 or FX for high-throughput applications.

In preparation for protein purification, bacterial cells expressing a polyhistidine- or HQ-tagged protein are lysed directly in culture using the provided FastBreak™ Cell Lysis Reagent^(a,b,c). The HisLink™ Resin is added directly to the lysate, mixed, and the polyhistidine- or HQ-tagged proteins bind in a matter of minutes. The samples are then transferred to a Filtration Plate. Unbound proteins are washed away, and the target protein is recovered by elution. Figure 1 describes protein purification using the HisLink™ 96 System. This system requires the use of the Vac-Man® 96 Vacuum Manifold or compatible vacuum manifold (Figure 2).

Advantages of the HisLink™ 96 System

- **Simple:** No centrifugation required – lysis buffer is added directly to cells in culture medium.
- **Quick:** No long lysozyme incubations are required for cell lysis.
- **Versatile:** Perform purification manually or on an automated platform.
- **Efficient:** Binding capacity of 1mg of polyhistidine-tagged protein per well.

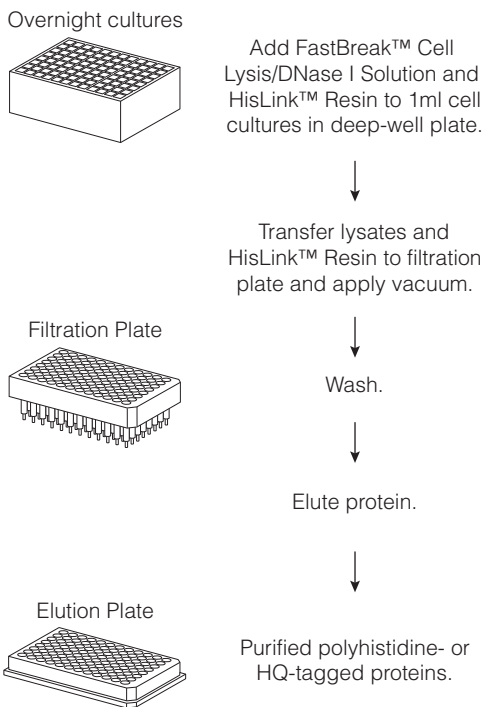


Figure 1. Flow diagram of polyhistidine- or HQ-tagged protein purification using the HisLink™ 96 Protein Purification System.

2. Product Components and Storage Conditions

Product	Size	Cat. #
HisLink™ 96 Protein Purification System	1 × 96 reactions	V3680

Each system contains sufficient reagents for 96 manual purifications from 1ml of bacterial culture. Includes:

- 15ml FastBreak™ Cell Lysis Reagent, 10X
- 1 vial DNase I
- 25ml HisLink™ Resin
- 110ml Binding/Wash Buffer
- 25ml Elution Buffer
- 1 Filtration Plate
- 1 Collection Plate

Product	Size	Cat. #
HisLink™ 96 Protein Purification System	5 × 96 reactions	V3681

Each system contains sufficient reagents for 480 manual purifications from 1ml of bacterial culture. Includes:

- 60ml FastBreak™ Cell Lysis Reagent, 10X
- 1 vial DNase I
- 2 × 50ml HisLink™ Resin
- 2 × 280ml Binding/Wash Buffer
- 125ml Elution Buffer
- 5 Filtration Plates
- 5 Collection Plates

Storage Conditions: Store all HisLink™ 96 reagents at 4°C. The plates may be stored at 4°C or at room temperature. Following reconstitution with water, DNase I should be stored in aliquots at -20°C. **FastBreak™ Cell Lysis Reagent** may form a precipitate at low temperature. If this occurs, warm the reagent to room temperature before use.

Available Separately

This product requires use of a Vac-Man® 96 Vacuum Manifold.

Product	Size	Cat. #
Vac-Man® 96 Vacuum Manifold	1 item	A2291

3. Polyhistidine- or HQ-Tagged Protein Purification Protocol

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.)

- Nuclease-Free Water (Cat.# P1195)
- Vac-Man® 96 Vacuum Manifold (Cat.# A2291)
- plate shaker (manual) or multichannel pipette
- wide-bore tips (E&K Scientific Cat.# 3502 - R96S)
- 96-well, deep-well plates (e.g., ABgene 2.2ml storage plate, Marsh Bio Products Cat.# AB-0932)
- 96-well plate mats (Phenix Research Products Cat.# M-0662)
- 96-well plate adhesive sealers (Eppendorf Cat.# E00-127-480)
- reservoir boats (Diversified Biotech Cat.# RESE-3000)
- NaCl or 5M NaCl solution for use with HQ-tagged proteins

3.A. Preparation of Cell Culture

Bacterial cultures can be grown in tubes, flasks or deep-well, 96-well plates (Marsh Bio Products Cat.# AB-0932). Grow the culture containing 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 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 this system. Cells do not need any centrifugation or freezing for this purification system.

3.B. Considerations When Adding Lysozyme

The HisLink™ 96 System is designed to lyse cells without the addition of lysozyme. Lysozyme, if added, will co-purify with your polyhistidine- or HQ-tagged protein unless 500mM NaCl is added to the wash buffer.

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

For the 1-plate system (Cat.# V3680):

1. 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.

- If you plan to use the entire plate (96 wells), add the entire DNase I dilution to 11ml of FastBreak™ Cell Lysis Reagent, 10X, and mix well. For fewer wells, smaller amounts of FastBreak™ Reagent/DNase I solution can be prepared using the same ratio of DNase I to FastBreak™ Reagent (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.

Wells	DNase I Solution	FastBreak™ Reagent	Table 1. FastBreak™ Reagent/DNase I Solution Volumes. Volumes for preparing the FastBreak™ Reagent/DNase I solution for sizes other than a full 96-well plate.
1	8µl	92µl	
8	67µl	733µl	
16	133µl	1,467µl	
24	200µl	2,200µl	

For the 5-plate system (Cat.# V3681):

- Add 275µl of Nuclease-Free Water to the vial of DNase I.
- Mix completely to dissolve the powder.
- Remove the DNase I solution from the vial and add it to 4.75ml of Nuclease-Free Water. Mix well.
- If it will not be used immediately, dispense 1ml aliquots of the DNase I dilution and store at -20°C.
Note: Once resuspended, the DNase I solution can be stored at -20°C for 6 months and is stable for 8 freeze-thaw cycles.
- For each 96-well plate, thaw one 1ml aliquot of DNase I solution at 22°C. Add 1ml of the thawed aliquot to 11ml of FastBreak™ Cell Lysis Reagent, 10X. Mix well before using. If less than a full plate is used, use the same ratio of DNase I to FastBreak™ Reagent (i.e., add 455µl of the DNase I solution to 5ml of FastBreak™ Reagent).

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.

3.D. Manual Polyhistidine- or HQ-Tagged Protein Purification

1. To 1ml of culture, add 100µl of the FastBreak™ Reagent/DNase I solution prepared in Section 3.C.

Note: Adding 200mM NaCl prior to the addition of the HisLink™ Resin may reduce nonspecific binding and improve binding of HQ-tagged proteins.

2. Resuspend the resin and allow time for the resin to settle before removing. Once the resin has settled, use wide-bore pipette tips to transfer 75µl of the HisLink™ Resin from the settled bed of resin to each well of the plate.

Note: For best results, pour the resin into a reservoir and allow it to settle. 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 of the resin from the reservoir or bottle.

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

3. Mix the sample. Shake for 30 minutes or periodically pipet or vortex over a 30-minute period.

Note: Binding will not be efficient if the resin is not mixed frequently during this time period. We recommend sealing the deep-well plate with a 96-well mat to prevent spills.

4. Place a Filtration Plate onto the vacuum manifold base (Figure 2, Panel A).

Note: If you wish to collect the flowthrough, place an empty deep-well plate on the manifold bed. On top of the deep-well plate place the manifold collar and insert the Filtration Plate onto the collar before transferring the lysate (Figure 2, Panel B).

5. Use a wide-bore pipette to transfer the lysate and resin to the Filtration Plate.

6. Cover unused Filtration Plate wells with an adhesive plate sealer.

7. Apply vacuum to the samples for 10 seconds.

8. If you collected the flowthrough, remove the Filtration Plate from the manifold collar and place the Filtration Plate onto the vacuum manifold base (Figure 2, Panel B).

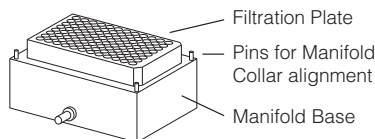
9. Add 250µl of the wash buffer to the wells of the Filtration Plate. Apply vacuum for 10 seconds.

Note: If there are still particles in the culture plate, add the wash buffer to the wells, resuspend the remaining particles and transfer them to the Filtration Plate. Apply vacuum for 10 seconds.

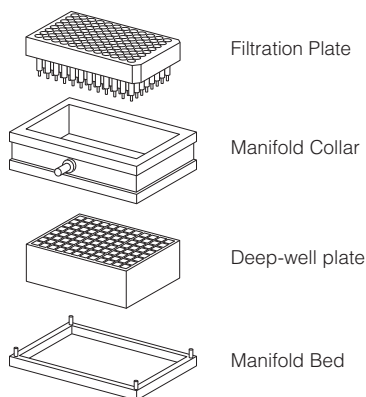
10. Repeat Step 9 three more times for a total of four washes.

11. Place the Filtration Plate onto a clean absorbent towel to remove any excess wash buffer from the ports located on the bottom of the plates.
12. Place the Collection Plate onto the manifold bed (Figure 2, Panel C).
13. Place the manifold collar on the Collection Plate, fitting it into the pins of the manifold bed.
14. Place the Filtration Plate onto the manifold collar. To prevent uneven flow or spattering, remove the vacuum hose from the port on the manifold collar. Reattach the vacuum hose at Step 16.
15. Add 200 μ l of the 1X Elution Buffer. Wait 3 minutes.
Note: HQ-tagged proteins may elute with a lower concentration of imidazole (50–100mM) compared to polyhistidine-tagged proteins.
16. Reattach the vacuum hose to the manifold collar.
17. Collect the eluate by applying a vacuum for 1 minute.
Note: Prior to gel analysis, mix samples thoroughly.

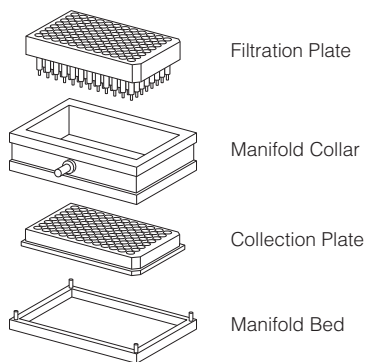
A. Protein Wash Apparatus



B. Flowthrough Collection



C. Elution Apparatus



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Figure 2. Diagram of the Vac-Man® 96 Vacuum Manifold with the HisLink™ 96 Protein Purification System. Diagram shows the manifold and plate combinations necessary to accomplish protein purification with the HisLink™ 96 Protein Purification System.

3.E. Automated Purification Using the Beckman Coulter Biomek® 2000 Robotic Platform

The manual protocol described in Section 3.D can be used as a guide to develop protocols for automated workstations. The protocol may require optimization depending on the instrument used. This system has been fully automated on the Beckman Coulter Biomek® 2000. For more information, please refer to the documentation provided with the BioWorks™ method. The BioWorks™ method may be downloaded at:
www.promega.com/automethods/

Materials to be Supplied by the User

The following is a list of Beckman Coulter parts and their corresponding part numbers that are required to automate the HisLink™ 96 Protein Purification System on a Biomek® 2000.

Table 2. Instrumentation Requirements for the Biomek® 2000 Workstation.

Description	Beckman Coulter Part #
Biomek® 2000 Workstation, 50/60Hz, 100–120V	609000
Biomek® 2000 Controller NT	609875
IBM Monitor	974571
BioWorks™ 3.2 for Beckman Coulter Computer	609983
Gripper Tool System for Biomek® 2000	609001
Worksurface Spill Tray	609077
MP200 Pipetting Tool	609025
Tip Rack Holder (2)	609121
Gray Labware Holder (4)	609120
Collar Holders	609736
Vacuum Valve Unit	609005
Vacuum Filtration Manifold Base	609670
36mm Vacuum Collar	609597
Elution Spacer	390792
Vacuum Regulator	609674
Tubing Kit, Filtration System	609676
Tubing Kit, Wash Unit	609687
Plastic Bottle, 4L	975796
Cap	975797
Reservoir Holder	372795
Quarter Single Reservoirs	372790
Quarter Vertical Reservoirs	372788

Table 3. Labware Requirements for the Biomek® 2000 Workstation.

Labware Requirements	Source
Wide-Bore 250µl Tips	Axygen Tips, VWR Cat.#. 47744-802
2.2ml deep-well plate	Marsh Bio Products Cat.# AB- 0932

1. Download the BioWorks™ method from:
www.promega.com/automethods
2. Prepare the Biomek® 2000 deck according to the instructions provided in the BioWorks™ method.
3. Place the deep-well plates containing the cell culture at the appropriate place on the Biomek® deck and start the BioWorks™ method.

4. General Considerations

Elution

Imidazole (50–1,000mM): For certain proteins and/or purification tags, elution efficiency may need to be optimized. Polyhistidine-tagged proteins can be eluted using 250–1,000mM imidazole. Polyhistidine tags containing less than six histidines typically require less imidazole for elution, while polyhistidine proteins containing more than six polyhistidines may require higher levels of imidazole. The HQ tag contains three histidines and three glutamines (HQQHQ) 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 may result in leaching of nickel, which may become quite significant at pH values below 4. These considerations also apply to HQ-tagged proteins.

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.

Reusing Resin

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

Adjuncts for Lysis or Purification

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

Additive	Concentration
HEPES, Tris or sodium phosphate buffers	≤100mM
NaCl	≤1M
β-mercaptoethanol	≤100mM
DIT	≤10mM
EDTA	≤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

<u>Symptoms</u>	<u>Causes and Comments</u>
Low amounts of or no protein recovered	<p data-bbox="466 310 883 363"><u>Protein expression may not be induced. Check concentration and stability of inducer (e.g., IPTG).</u></p> <p data-bbox="466 379 883 512"><u>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).</u></p> <p data-bbox="466 528 883 627"><u>Protein may be expressed in low amounts. Try different temperatures during induction. Check by Western blotting if using polyhistidine tag. Check by an activity assay if using HQ tag.</u></p> <p data-bbox="466 644 883 702"><u>Confirm that the sequence of the clone is correct by sequencing.</u></p> <p data-bbox="466 718 883 792"><u>Protein may be expressed in inclusion bodies. Check the lysate, pellet or flowthrough for the presence of insoluble protein of interest.</u></p> <p data-bbox="466 809 883 883"><u>Cells not lysing properly. Be sure to use the recommended volume of FastBreak™ Cell Lysis Reagent, 10X.</u></p> <p data-bbox="466 900 883 982"><u>Particles and lysate are not well mixed. Mix frequently for 30 minutes using a shaker or vortex mixer.</u></p> <p data-bbox="466 999 883 1073"><u>Protein may have metal binding domain. Try eluting with a higher concentration of imidazole or with acidic conditions such as citrate.</u></p>
Co-elution of contaminating proteins	<p data-bbox="466 1090 883 1172"><u>There may be an interacting protein in the cell. Add 0.5M–1M NaCl (final concentration) to the wash buffer.</u></p> <p data-bbox="466 1189 883 1291"><u>Add protease inhibitors to the FastBreak™ Reagent/DNase I solution, wash and elution solutions to prevent possible protein degradation.</u></p>

Symptoms	Causes and Comments
Lysate not clearing the Filtration Plate	Incorrect amount of DNase I may have been added. Check that the DNase I was diluted properly (Section 3.C).
	DNase I no longer active. Try using a fresh aliquot of DNase I. Store resuspended DNase I in aliquots at -20°C.
	Culture O.D. ₆₀₀ exceeds system recommendations. Adjust the cell concentration or add more DNase I.
	Unused wells were not covered. Seal any unused wells to ensure good vacuum efficiency.
Wash solution not clearing	Poor vacuum efficiency. Check vacuum connection to the Filtration Plate to be sure that the hoses are connected properly.
	Unused wells were not covered. Seal any unused wells to ensure good vacuum efficiency.

6. Composition of Buffers and Solutions

Binding/Wash Buffer (pH 7.5)

100mM HEPES
10mM imidazole

Elution Buffer (pH 7.5)

100mM HEPES
500mM imidazole

7. References

1. Hochuli, E. (1990) Purification of recombinant proteins with metal chelate adsorbent. *Genetic Engineering News* **12**, 87-98.

8. Related Products

Product	Size	Cat. #
Vac-Man® 96 Vacuum Manifold	1 each	A2291
HisLink™ Protein Purification Resin	50ml	V8821
HisLink™ Spin Protein Purification System	25 reactions	V1320
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 × 20cm 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 MKHQHQQAIA 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® Vector Systems for cloning, refer to the *Flexi® Vector System Technical Manual #TM254* or visit: www.promega.com

ⒶPatent Pending.

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