



MagneHis™ Protein Purification System

Technical Manual No. 060

INSTRUCTIONS FOR USE OF PRODUCTS V8500 AND V8550.
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I. Description

The MagneHis™ Protein Purification System^(a,b) provides a simple, rapid and reliable method for the purification of polyhistidine-tagged, expressed proteins (Figure 1). Paramagnetic precharged nickel particles (MagneHis™ Ni-Particles) are used to isolate His-tagged protein directly from a crude cell lysate using either a manual or automated procedure. Using the manual protocol, His-tagged protein can be purified on a small scale using less than 1ml of culture or on a large scale using more than 1L of culture. Samples can also be processed using a robotic platform such as the Beckman Biomek® 2000 or FX for high-throughput applications.

Bacterial cells containing a His-tagged protein are lysed using the provided MagneHis™ Cell Lysis Reagent, and MagneHis™ Ni-Particles are added to the lysate. His-tagged proteins bind to the Ni-Particles in a matter of minutes. Unbound proteins are washed away, and the target protein is recovered by elution with imidazole (Figure 2). His-tagged proteins can be purified under nondenaturing conditions or in the presence of either 6M urea or guanidine-HCl.

The MagneHis™ Protein Purification System has the following features:

- **Simple:** No centrifugation or vacuum is required once the cells are lysed.
- **Flexible:** MagneHis™ Ni-Particles are compatible with a variety of common buffers.
- **Quick:** No long incubations with lysozyme are required for cell lysis.
- **Scalable:** Volumes can be adjusted to correspond to the amount of material to be purified, 1ml to 1 liter.
- **Efficient:** Binding capacity is up to 1mg of His-tagged protein per 1ml of MagneHis™ Ni-Particles.
- **Versatile:** Purification can be performed manually or by using an automated platform.
- **Convenient:** Complete system that includes all necessary components, including a unique lysis buffer.

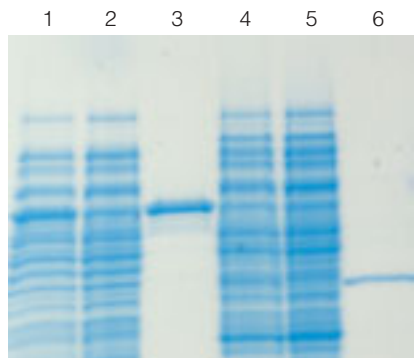


Figure 1. Purification of 6X His-tagged fusion proteins using the MagneHis™ Protein Purification System. Lane 1, bacterial cell lysate expressing 6X His-tagged firefly luciferase. Lane 2, column flowthrough of the lysate. Lane 3, eluted 6X His-tagged firefly luciferase. Lane 4, bacterial cell lysate expressing 6X His-tagged *Renilla* luciferase. Lane 5, column flowthrough of the lysate. Lane 6, eluted 6X His-tagged *Renilla* luciferase.

II. Product Components

Product	Size	Cat.#
MagneHis™ Protein Purification System	2ml	V8500

Each system contains sufficient reagents for 65 manual purifications each using 1ml of lysate. Includes:

- 50ml MagneHis™ Binding/Wash Buffer
- 10ml MagneHis™ Elution Buffer
- 14ml MagneHis™ Cell Lysis Reagent
- 2ml MagneHis™ Ni-Particles
- 1 Protocol

Product	Size	Cat.#
MagneHis™ Protein Purification System	10ml	V8550

Each system contains sufficient reagents for 325 manual purifications each using 1ml of lysate. Includes:

- 250ml MagneHis™ Binding/Wash Buffer
- 50ml MagneHis™ Elution Buffer
- 70ml MagneHis™ Cell Lysis Reagent
- 10ml MagneHis™ Ni-Particles
- 1 Protocol

Storage Conditions: Store all MagneHis™ Protein Purification System components at 4°C. **Do not freeze the MagneHis™ Ni-Particles.**

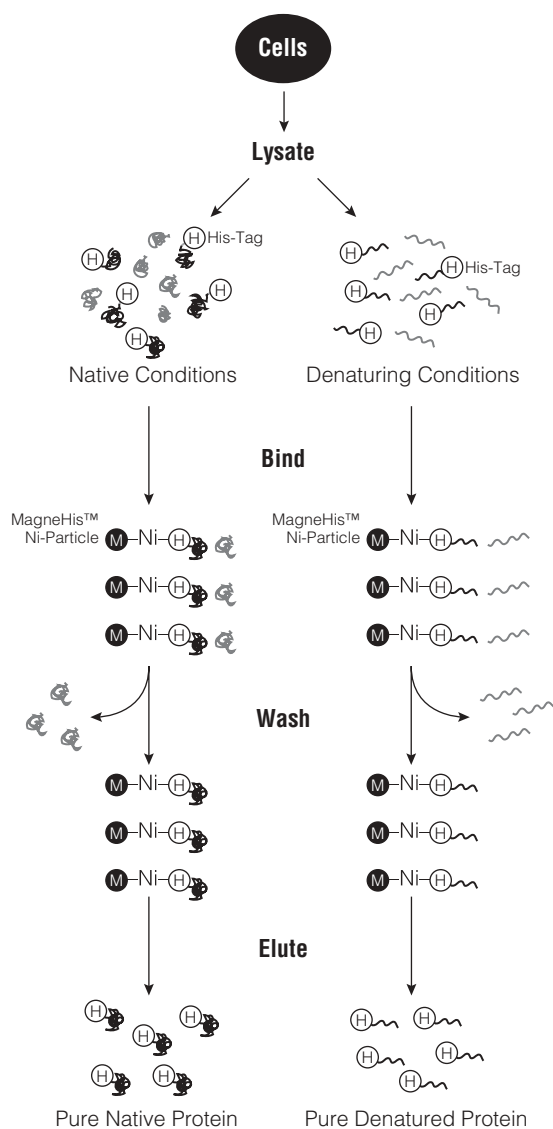


Figure 2. Diagram of the MagneHis™ Protein Purification System protocol.

Note: This protocol requires a magnetic stand that will accommodate small-scale purifications using microcentrifuge tubes or large-scale purifications using 15ml or 50ml conical tubes. Promega supplies Magnetic Separation Devices that can be used to hold 1–12 tubes.



We have found that the MagneHis™ Cell Lysis Reagent efficiently lyses the *E. coli* strains BL21(DE3)pLysS and JM109. Maximal lysis in some strains may require the addition of lysozyme. Refer to Section III.G, comment 7, for details regarding the use of lysozyme.

Note: The MagneHis™ Cell Lysis Reagent was designed for use with the MagneHis™ System. Other commercially available lysis reagents may not work properly with this system.

Note: An Experienced User's Protocol can be found at the end of this Technical Manual.

III. MagneHis™ Protein Purification Protocol

Materials to be Supplied by the User

- 37°C incubator for flasks/tubes
- shaker
- magnetic separation stand
- centrifuge

A. Preparation of Cells

Bacterial cultures can be grown in tubes, flasks or 2.2ml, 96-well plates (Marsh Bio Products Cat.# AB-0932). Grow the culture containing the appropriate His-tag fusion protein to an O.D.₆₀₀ between 0.4 and 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. We recommend that cell cultures have a final O.D.<9. Pellet cells by centrifuging at 16,000 × *g* for 10 minutes and carefully remove the supernatant. The cell pellet can be frozen at –70°C or the cells can be lysed immediately.

B. Cell Lysis

Cells may be lysed using the MagneHis™ Cell Lysis Reagent or by other methods.

Lysis using MagneHis™ Cell Lysis Reagent: For every 1ml of original culture volume, add 200µl of MagneHis™ Cell Lysis Reagent to the cell pellet (Table 1) and incubate with shaking for 10 minutes at room temperature. After the cells are lysed, the lysate can be used directly in the purification procedure. **There is no additional centrifugation step necessary to remove cellular debris.**

Table 1. Amount of MagneHis™ Cell Lysis Reagent for Different Culture Volumes.

Culture Volume	Cell Lysis Reagent Volume
1ml	200µl
10ml	2ml
50ml	10ml
100ml	20ml
1,000ml	200ml

Other lysis methods: Cells may be lysed by sonication, French press or other similar methods. For each of these methods, resuspend the cells in 100µl of MagneHis™ Binding/Wash Buffer per 1ml of culture before cell lysis. Lyse the cells and clear the lysate by centrifugation at 16,000 × *g* for 10 minutes. Proceed with the purification procedure. **See comment 7 in Section III.G before using lysozyme to lyse the cells.**

Note: We recommend using protease inhibitors during cell lysis. Addition of protease inhibitors such as 1mM PMSF to cell lysates does not inhibit the binding or elution of His-tag proteins from MagneHis™ Ni-Particles. When preparing cell lysates from high-density cultures, DNase and RNase can be added to a final concentration of 20µg/ml each and incubated for 10 minutes at room temperature prior to adding the MagneHis™ Ni-Particles.

C. Small-Scale Purification of His-Tag Proteins Under Native Conditions (1ml cultures)

Small-scale purification of His-tag proteins using the MagneHis™ System is useful for screening multiple clones for expression, optimizing expression conditions (temperature, media, host strain, etc.), and the initial screening of mutant clones. The entire purified sample (including the MagneHis™ Ni-Particles) can be analyzed on a SDS-polyacrylamide gel to identify low expressing protein, reducing the need for Western blot analysis (see Note 3 below).

If the His-tagged protein is located in an inclusion body, use the denaturing conditions described in Section III.E.

1. Vortex the MagneHis™ Ni-Particles to a uniform suspension.
2. Add 30µl MagneHis™ Ni-Particles to 200µl of cell lysate (equivalent to 1ml of culture).
3. Mix by pipetting up and down approximately 10 times and incubate for 2 minutes at room temperature.
4. Place the tube in the appropriate magnetic stand for approximately 30 seconds. Allow the MagneHis™ Ni-Particles to be captured by the magnet. Carefully remove the supernatant with a pipette.
5. Remove tube from the magnet. Add 150µl of MagneHis™ Binding/Wash Buffer to the MagneHis™ Ni-Particles and mix by pipetting. Make sure that particles are resuspended well.
6. Place the tube in the appropriate magnetic stand for approximately 30 seconds. Allow the MagneHis™ Ni-Particles to be captured by the magnet, and carefully remove supernatant with a pipette.
7. Repeat the wash step 2 times for a total of 3 washes.
8. Add 100µl of MagneHis™ Elution Buffer and mix by pipetting. Incubate for 1–2 minutes at room temperature. Place in a magnetic stand. Allow the MagneHis™ Ni-Particles to be captured by the magnet and remove supernatant containing the purified protein using a pipette. Analyze the samples for expression of the fusion protein by SDS-PAGE or by functional assay.

Notes

1. A second elution using another 100µl of MagneHis™ Elution Buffer may increase the final yield.
2. To elute the purified protein in a more concentrated form, elute with 50µl MagneHis™ Elution Buffer. Do not increase the amount of MagneHis™ Ni-Particles used when eluting in a smaller volume or the yield will be reduced.
3. Step 8 can be omitted and the sample containing the MagneHis™ Ni-Particles can be used for SDS-PAGE analysis. Add 25–50µl of water to the washed MagneHis™ Ni-Particles. Add 4X SDS gel-loading buffer to a final concentration of 1X and load sample directly onto a SDS-polyacrylamide gel.

Note: The MagneHis™ Protein Purification System can be used with eukaryotic expression systems. Contact Promega Technical Services for more information.


Note: Purify secreted His-tagged proteins directly from cleared culture medium by adding MagneHis™ Ni-Particles to the medium based on the level of expression. Adjust the pH of the medium to pH 7.5 before adding the Ni-Particles.



See alternate elution conditions in Section III.F.

Note: Purify secreted His-tagged proteins directly from cleared culture medium by adding MagneHis™ Ni-Particles to the medium based on the level of expression. Adjust the pH of the medium to pH 7.5 before adding the Ni-Particles.

Note: Use tubes that can accommodate the volume of the MagneHis™ Ni-Particles and MagneHis™ Binding/Wash Buffer required for the purification. The tubes must be compatible with a magnetic separation stand. It may be necessary to use multiple tubes.

 See alternate elution conditions in Section III.F.

D. Large-Scale Purification of His-Tag Proteins Under Native Conditions (1–1,000ml cultures)

1. Vortex the MagneHis™ Ni-Particles to a uniform suspension.
2. Adjust the amount of MagneHis™ Ni-Particles used according to the culture volume and the amount of expressed protein using Table 2 as a guideline. A rough estimate of the expression level can be determined using functional assays or by SDS-PAGE. Compare your induced samples to control cells without the His-tag vector or uninduced cells.

Alternatively, optimize the amount of MagneHis™ Ni-Particles to use by performing a titration experiment using 10, 20, and 30µl of MagneHis™ Ni-Particles in the small-scale protocol (Section III.C). Analyze the results by SDS-PAGE and identify the lowest amount of Ni-Particles that produces good results. Scale up the volume of Ni-Particles to use for the large-scale purification based on the culture volume.

Table 2. The Amount of MagneHis™ Ni-Particles Used Depending on Expression Level and Culture Volume.

Level of His-tag Protein Expressed (mg per liter)	Culture Volume (ml)			
	1	10	100	1,000
30	30µl	300µl	3ml	30ml
20	20µl	200µl	2ml	20ml
10	10µl	100µl	1ml	10ml
1	N/A	N/A	0.1ml	1ml

3. Gently mix the MagneHis™ Ni-Particles with the cell lysate by inverting the tube at room temperature for 2 minutes.
4. Place the sample in the appropriate magnetic stand and allow the MagneHis™ Ni-Particles to be captured by the magnet. Large-scale purification may require several minutes for the particles to be captured by the magnet. Carefully pour off supernatant.
5. Remove the tube from the magnet. Wash the Ni-Particles with MagneHis™ Binding/Wash Buffer. For each wash, use 5X the volume of MagneHis™ Ni-Particles used. (For example, if 1ml of MagneHis™ Ni-Particles was used for purification, use 5ml of MagneHis™ Binding/Wash Buffer for each washing.)
6. Place tube in a magnetic stand for approximately 1 minute. Allow the MagneHis™ Ni-Particles to be captured by the magnet, and carefully remove the supernatant.
7. Repeat the wash step 2 times for a total of 3 washes.
8. Adjust the volume of MagneHis™ Elution Buffer according to the amount of Ni-Particles used. Use 100µl of Elution Buffer per 30µl of Ni-Particles. Mix the MagneHis™ Elution Buffer with the MagneHis™ Ni-Particles and incubate for 1–2 minutes at room temperature. Place the tube into a magnetic stand. Allow the MagneHis™ Ni-Particles to be captured by the magnet, and carefully remove the supernatant with a pipette. If you are using >100µl of Ni-Particles, multiple elution steps will maximize your yield.

E. Denaturing Conditions

Since the interaction of His-tag fusion proteins and MagneHis™ Ni-Particles does not depend on tertiary structure, fusion proteins can be captured and purified using denaturing conditions. If the His-tag fusion is found in inclusion bodies, solubilize by adding a strong denaturant such as guanidine hydrochloride or urea.

To determine if the His-tagged protein is located in inclusion bodies, perform the lysis step using MagneHis™ Cell Lysis Reagent (Section III.B). Pellet the cellular debris by centrifugation and test the supernatant and the pellet for the His-tagged protein by enzyme assay or Western analysis. If the majority of the His-tagged protein is associated with the pellet, use denaturing conditions for isolation.

To isolate His-tag fusion proteins under denaturing conditions, perform the large- or small-scale purification procedure using MagneHis™ Binding/Wash Buffer and MagneHis™ Elution Buffer containing 6M urea or guanidine-HCl. **For the lysis step (Section III. B), use MagneHis™ Binding/Wash Buffer with 6M urea or guanidine-HCl instead of MagneHis™ Cell Lysis Reagent.** If the tagged protein contains disulfide bridges, 100mM DTT can be used in both the Binding/Wash and Elution Buffers.

F. Alternative Elution Conditions

For certain applications, alternative elution conditions may be required (e.g., mass spectrometry analysis). Elution conditions may be optimized or altered as needed (Table 3).

Table 3. Alternative Elution Conditions.

Reagent	Effect
MagneHis™ Elution Buffer (500mM imidazole)	Decreasing volume of Elution Buffer will concentrate protein.
MagneHis™ Elution Buffer (500mM imidazole)	In some cases, a second elution may release more His-tagged protein.
0.5–1M imidazole	Increasing concentration of imidazole may elute more His-tagged protein.
100mM EDTA	Removes the Ni-His-tagged protein complex from paramagnetic particle.
0.1% trifluoroacetic acid (TFA) with or without acetonitrile	Can be used to elute protein from the Ni-Particle and is useful in mass spectrometry analysis (see below).
1M sodium citrate (pH 4–6)	Decreasing pH will elute protein. Neutralizing the eluate immediately after elution helps retain the function of the protein.
MagneHis™ Elution Buffer diluted with an equal volume of water or Binding/Wash Buffer (250mM imidazole)	Reduces the inhibitory effect of imidazole in downstream applications.

Mass Spectrometry Analysis

If the purified protein is to be used for mass spectrometry analysis, imidazole elution may be replaced with trifluoroacetic acid (TFA).

1. After washing the MagneHis™ Ni-Particles with MagneHis™ Binding/Wash

Buffer, wash the Ni-Particles twice with 150µl of 10mM ammonium acetate, pH 7.5 or 30% ethanol.

2. Elute with 100µl of 0.1% TFA.
3. Dry sample in a Speed Vac® concentrator or air-dry.
4. Resuspend the sample in the solvent or buffer that is will be used for mass spectrometry analysis.

G. General Considerations

1. Proteins expressed for purification with this system should have at least five to six consecutive histidine residues located on the C or N terminus.
2. The MagneHis™ Binding/Wash Buffer contains 10mM imidazole to prevent nonspecific binding to the MagneHis™ Ni-Particles.
3. The level of washing done will determine the level of background proteins observed.
4. The MagneHis™ Cell Lysis Reagent has been tested with bacterial strains BL21(DE3)pLysS and JM109.
5. MagneHis™ Ni-Particles have been evaluated for compatibility with several common buffer components (Table 4).

Table 4. Tolerance of MagneHis™ Ni-Particles Using Alternative Buffers.

Reagent	Concentration
β-mercaptoethanol	≤100mM
cationic detergents	1%
DTT	≤100mM
EDTA	≤5mM
glycerol	≤20%
guanidine-HCl	≤7M
NaCl	≤1M
Tris, MOPS, sodium phosphate, potassium phosphate	100mM
Triton® detergent	1%
Tween® 20	0.05%
urea	≤6M

6. MagneHis™ Ni-Particles should be stored and handled carefully to avoid contamination. Always use new pipette tips. Do not store MagneHis™ Ni-Particles adjacent to culture plates, especially when working with yeast cultures.
7. Lysozyme binds to the MagneHis™ Ni-Particles, so lysozyme used to lyse the cells will elute with the fusion protein. To prevent binding of lysozyme to the Ni-Particles, add 0.5M NaCl to the MagneHis™ Binding/Wash Buffer. Lysozyme will produce a 12.5kD band on an SDS-polyacrylamide gel.
8. Purified His-tagged protein can be quantitated using the standard methods such as Bradford or BCA, but the imidazole in the Elution Buffer may inhibit these assays. Either dialyze the sample or dilute to the optimal imidazole concentration for the protein quantitation reagent used.

9. If you intend to repurify a sample that has already been through MagneHis™ purification, you must reduce the concentration of imidazole in the sample by dilution or dialysis. Dilute the sample with MagneHis™ Binding/Wash Buffer or 100mM HEPES (pH 7.5) to a final concentration of 10mM imidazole. Alternatively, dialyze the sample with several changes of 100mM HEPES (pH 7.5).
10. We have used MagneHis™ Ni-Particles to purify His-tagged proteins generated in vitro by *E. coli* S30 lysate or wheat germ extract. Hemoglobin copurifies with the His-tagged protein when isolating His-tagged proteins from rabbit reticulocyte-based in vitro translation systems. For additional details, contact Technical Services.



A common reason for the dialyzed protein not to bind to the Ni-Particles is insufficient dialysis. If the dialyzed protein does not bind to the Ni-Particles, repeat dialysis or dilute with HEPES.

IV. Isolation of His-Tag Fusion Proteins in a 96-Well Plate Using the Beckman Biomek® 2000 Robotic Platform

The manual protocol described in Section III can be used as a guide to develop protocols for automated workstations. The protocol may require optimization depending on the instrument used. Promega has an ongoing effort to develop procedures for different automated platforms. As new protocols are developed, information will be posted to the Promega website (www.promega.com/automethods).

This system has been fully automated on the Beckman 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

- tabletop centrifuge capable of 2,000 × *g*
 - Deep-well 96-well plates (e.g., Marsh Bio Products Cat.# AB-0932)
 - MagnaBot® 96 Magnetic Separation Device (Promega Cat.# V8151)
 - Twinivour low volume quarter vertical reservoir (Acme-Automation Cat.# C5001)
 - Biomek® 2000 workstation, 50/60Hz, 100–120V (Beckman Part# 609000)
 - Biomek® 2000 controller NT (Beckman Part# 609875)
 - BioWorks™ 3.2 for Beckman Coulter computer (Beckman Part# 609983)
 - Biomek® 2000 left side module (Beckman Part# 609048)
 - MP200 pipetting tool (Beckman Part# 609025)
 - gripper tool system for Biomek® 2000 (Beckman Part# 609001)
 - DPC MicroMix® 5 shaker (Beckman Part# 380560)
 - DPC MicroMix® 5 Integration kit (Beckman Part# 380561)
 - tip rack holder (3) (Beckman Part# 609121)
 - gray labware holder (5) (Beckman Part# 609120)
 - reservoir holder (1) (Beckman Part# 372795)
 - quarter single reservoirs (2) (Beckman Part# 372790)
 - quarter vertical reservoir (1) (Beckman Part# 372788)
 - automated protocol
1. Download the BioWorks™ method from: www.promega.com/automethods
 2. Prepare the BioMek® deck according to the instructions provided in the Bioworks™ method.
 3. Centrifuge the 96-well plates containing cell cultures at 2,000 × *g* for 15 minutes.

4. Carefully remove the supernatant.
5. Place the 96-well plates containing the cell pellets at the appropriate place on the BioMek[®] deck and start the Bioworks[™] method.

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

V. Troubleshooting

Symptoms	Possible Causes	Comments
Protein is not expressed	Sequence or orientation is not correct	Confirm the clone by sequencing.
	Cells are not induced	Use the correct inducers, such as IPTG for T7-based expression in BL21(DE3) cells.
	Expressed protein is not 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 expressed in low amount	Check by Western blotting. Try different temperatures during induction (16–37°C).
Protein expressed in inclusion bodies	Protein expressed in low amount	Check the lysate pellet or flowthrough for the presence of insoluble protein.
	Protein expressed in inclusion bodies	Check the lysate pellet or flowthrough for the presence of insoluble protein.
Protein isolated in low quantity or not eluting from resin	Protein may have a metal binding domain	Elute with higher concentration of imidazole (e.g., 1M) or with acidic conditions such as TFA or citrate.
His-tag protein not binding to the resin	Sequence is not correct	Confirm the clone by sequencing.
	Protein degradation	Add protease inhibitors.
	Buffer pH is not correct	Problematic if trying to purify proteins secreted into the media. Adjust pH to 7.5 before binding.
Expressed protein disappears after purification	Protease contamination	Add protease inhibitors to the Lysis, Binding/Wash and Elution Buffers.
Cell Lysis Reagent does not release the His-tag protein, but sonicated sample contains the protein	Protein of interest may be a DNA or RNA binding protein	Treat the lysate with DNase or RNase during or after lysis.
	Protein of interest is a membrane protein or is attached to bacterial membranes specifically or non-specifically	Solubilize the protein with suitable detergent. If problem still exists, sonicate the sample.
Cells are not lysing with the MagneHis [™] Cell Lysis Reagent	Added wrong volume of Cell Lysis Reagent	Use recommended volume of Cell Lysis Reagent.
	Some media components can inhibit cell lysis	Remove all of the media from the pellet.
A few proteins are coeluted with your protein, even after extensive washing	Specific protein degradation	Add protease inhibitors to the Binding/Wash and Elution Buffers.
	There is an interacting protein in the host cell	Add 0.5–1M NaCl to washing step.

V. Troubleshooting (continued)

Symptoms	Possible Causes	Comments
Eluted proteins form dimers or aggregates	Lone cysteine is present in His-tagged protein	Add 100mM DTT to the Lysis, Binding/Wash and Elution Buffers.
	Some proteins may aggregate during the denaturation step	Addition of 0.5–1M NaCl may prevent nonspecific interaction. Denature at 50°C for 15 minutes or 37°C for 30 minutes before SDS-PAGE analysis.
Problem with downstream applications	Inhibition by imidazole	Elute using one of the alternative methods in Table 3.
High background	Incomplete washing	Wash Ni-Particles 3X with at least 5 volumes of Binding/Wash Buffer for each wash. Add 0.5–1M NaCl in the binding and washing steps.
After the binding step, Ni-Particles are clumping or cannot be fully magnetized	Cells not lysing properly	See “Cells are not lysing with the MagneHis™ Cell Lysis Reagent”.
	Possible contamination	Check Ni-Particles for contamination by plating some of the Ni-Particle storage solution on an LB plate and incubating at 37°C overnight.
	Nucleic acid contamination	Add DNase and RNase.
Fusion proteins are still present in the flowthrough	Did not use enough Ni-Particles	Increase the amount of Ni-Particles used.
Fusion protein is active after lysis but loses activity after elution	Inhibition by imidazole	Elute with lower concentration of imidazole. Elute under low pH conditions or using EDTA.
Purified protein precipitates during dialysis	High imidazole concentration	Elute using citrate or EDTA as described in Table 3.

VI. Appendix

A. O.D. Calculation

O.D.₆₀₀ = 10 × O.D.₆₀₀ of 1ml of a 1:10 dilution of the culture (diluted in medium) measured in a 1cm path length cuvette.

B. Composition of Buffers and Solutions

4X SDS gel-loading buffer

0.24M Tris-HCl (pH 6.8)
 2% SDS
 3mM bromophenol blue
 50.4% glycerol
 0.4M dithiothreitol

SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should be added from a 1M stock just before the buffer is used.

MagneHis™ Binding/Wash Buffer (pH 7.5)

100mM HEPES
 10mM imidazole

MagneHis™ Elution Buffer (pH 7.5)

100mM HEPES
 500mM imidazole

C. Related Products

Product	Size	Cat.#
JM109 Competent Cells, >10 ⁸ cfu/μg*	1ml	L2001
BL21(DE3)pLysS Competent Cells ^(c)	1ml	L1191
Bacterial Strain JM109(DE3), Glycerol Stock	500μl	P9801
Factor Xa Protease	50μg	V5581
Sequencing Grade Modified Trypsin*	100μg	V5111
Broad Range Protein Molecular Weight Markers	100 lanes	V8491
Gel Drying Kit, 17.5 × 20cm capacity	1 kit	V7120

*For Laboratory Use

Product	Size	Cat.#
MagneHis™ Ni-Particles	2ml	V8560
	10ml	V8565

(a) Patent Pending.

(b) Some applications in which this product may be used 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.

(c) Usage restrictions apply to Bacterial Strains JM109(DE3) and BL21(DE3)pLysS, to the following Promega products that include these bacterial strains (pGEMEX[®]-1 and pGEMEX[®]-2 Vectors) and to any derivatives thereof.

Usage Restrictions for the T7 Expression System

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patents and patent applications assigned to Brookhaven Science Associates, LLC (BSA). This technology, including bacteria, phage and plasmids that carry the gene for T7 RNA polymerase, is to be used for academic or nonprofit laboratory or licensed commercial research purposes only. By accepting or using the T7 expression technology you agree to be bound by the following conditions set forth by BSA. The initial purchaser may refuse to accept the conditions of this notice by returning this product and the enclosed materials to Promega unused.

Academic and NonProfit Laboratories

No materials that contain the cloned gene for T7 RNA polymerase may be distributed further to third parties outside of your laboratory unless the recipient receives a copy of this assurance notice and agrees to be bound by its terms. This limitation applies to Bacterial Strains JM109(DE3) and BL21(DE3)pLysS, to the following Promega products that include these bacterial strains (pGEMEX[®]-1 and pGEMEX[®]-2 Vectors) and to any derivatives thereof.

Commercial Laboratories

A license is required for any commercial use of the T7 expression system, including use of the T7 system for research purposes or for production purposes by any commercial entity. Information about commercial licenses may be obtained from the Licensing Office, Brookhaven National Laboratory, Upton, NY 11973, Telephone: 631-344-7134, FAX: 631-344-3729.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

MagneHis™ Protein Purification System: *Experienced User's Protocol*

This quick protocol is intended as an easy-to-follow reminder for experienced users. Please follow the complete protocol (Section III) the first time you use the MagneHis™ Protein Purification System.

Preparation of Cells (Section III.A)	Grow the culture containing the appropriate His-tag fusion protein to an O.D. ₆₀₀ between 0.4 and 0.6, then induce protein expression. Cultures should have a final O.D. ₆₀₀ < 9. Pellet cells by centrifugation and carefully remove the supernatant. The cell pellet can be frozen at -70°C or the cells can be lysed immediately.
Cell Lysis (Section III.B)	For every 1ml of original culture volume, add 200µl of MagneHis™ Cell Lysis Reagent to the cell pellet and incubate with shaking for 10 minutes at room temperature. We strongly recommend using protease inhibitors during cell lysis.
Small-Scale Purification (1ml cultures) (Section III.C)	<ol style="list-style-type: none"> 1. Vortex the MagneHis™ Ni-Particles to a uniform suspension. 2. Add 30µl MagneHis™ Ni-Particles to 200µl of cell lysate. 3. Mix by pipetting and incubate for 2 minutes at room temperature. 4. Place the tube in the appropriate magnetic stand for approximately 30 seconds. Allow the MagneHis™ Ni-Particles to be captured by the magnet. Carefully remove the supernatant. 5. Remove the tube from the magnet. Add 150µl of MagneHis™ Binding/Wash Buffer to the MagneHis™ Ni-Particles and mix by pipetting. Make sure that particles are resuspended well. 6. Place the tube in the appropriate magnetic stand for approximately 30 seconds. Allow the MagneHis™ Ni-Particles to be captured by the magnet and carefully remove the supernatant with a pipette. 7. Repeat the wash step 2 times for a total of 3 washes. 8. Add 100µl of MagneHis™ Elution Buffer and mix by pipetting. Incubate for 1–2 minutes at room temperature. Place in a magnetic stand. Allow the MagneHis™ Ni-Particles to be captured by the magnet, and remove the supernatant containing the purified protein using a pipette.
Large-Scale Purification (1–1,000ml cultures) (Section III.D)	<ol style="list-style-type: none"> 1. Vortex the MagneHis™ Ni-Particles to a uniform suspension. 2. Adjust the amount of MagneHis™ Ni-Particles required according to the culture size and amount of expressed protein using Table 2 as a guideline. Gently mix the MagneHis™ Ni-Particles with the cell lysate at room temperature for 2 minutes. 3. Place the sample in the appropriate magnetic stand and allow the MagneHis™ Ni-Particles to be captured by the magnet. Large-scale purification may require several minutes for the particles to be captured by the magnet. Carefully pour off supernatant. 4. Remove the tube from the magnet. Wash the Ni-Particles with MagneHis™ Binding/Wash Buffer. For each wash, use 5X the volume of MagneHis™ Ni-Particles used. 5. Place the tube in a magnetic stand for approximately 1 minute. Allow the MagneHis™ Ni-Particles to be captured by the magnet, and carefully remove the supernatant. 6. Repeat wash step 2 times for a total of 3 washes. 7. Use 100µl of Elution Buffer per 30µl of Ni-Particles. Incubate for 1–2 minutes at room temperature. Place into a magnetic stand. Allow the MagneHis™ Ni-Particles to be captured by the magnet, and carefully remove the supernatant containing the purified protein with a pipette.