

TECHNICAL MANUAL

MagneHis™ Protein Purification System

Instructions for use of Products
V8500, V8550, V8560 and V8565



MagneHis™ Protein Purification System

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

1. Description	1
2. Product Components and Storage Conditions	4
3. MagneHis™ Protein Purification Protocol	5
A. Preparation of Bacterial Cells	5
B. Bacterial Cell Lysis	5
Method 1: Lysis of Pelleted Bacterial Cells	5
Method 2: Direct Lysis of Bacterial Cell Culture.....	6
C. Purification of Polyhistidine- or HQ-Tagged Proteins from 1ml Bacterial Culture Using MagneHis™ Ni-Particles	6
D. Preparation of Insect or Mammalian Cells.....	7
E. Lysis and Purification of Polyhistidine- or HQ-Tagged Proteins from 1ml Cultured Insect or Mammalian Cells.....	7
F. Purification of Polyhistidine- or HQ-Tagged Proteins from 1ml Insect or Mammalian Cell Culture Medium	9
G. Guidelines For Using Denaturing Conditions.....	10
H. Alternative Elution Conditions	11
I. General Considerations	12
4. Isolation of Polyhistidine- or HQ-Tagged Fusion Proteins on Automated Platforms	13
5. Troubleshooting	13
6. References	16
7. Appendix	16
A. O.D. Calculation	16
B. Composition of Buffers and Solutions	16
C. Related Products.....	17

1. Description

The MagneHis™ Protein Purification System^(a,b,c) provides a simple, rapid and reliable method for the purification of polyhistidine- (Figure 1, reference 1) or HQ-tagged proteins. Paramagnetic precharged nickel particles (MagneHis™ Ni-Particles^(a)) are used to isolate polyhistidine- or HQ-tagged protein directly from a crude cell lysate of bacterial, insect or mammalian cells or from culture



1. Description (continued)

medium using either a manual or automated procedure. Using the manual protocol, polyhistidine- or HQ-tagged protein can be purified using 1ml of culture of up to 6 O.D.₆₀₀ of bacterial cells, or 2×10^6 insect or mammalian cells. Samples can also be processed using a robotic platform for high-throughput applications. 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.

Bacterial, insect or mammalian cells containing a polyhistidine- or HQ-tagged protein can be lysed directly in the culture medium using the provided FastBreak™ Cell Lysis Reagent, 10X^(a,b,c), or by adding 1X FastBreak™ Cell Lysis Reagent to cell pellets. MagneHis™ Ni-Particles are then added to the lysate. In addition, tagged proteins secreted into the medium of insect or mammalian cells can be purified without interference from serum in the medium. Polyhistidine- or HQ-tagged proteins bind to the MagneHis™ Ni-Particles in a matter of minutes. Unbound proteins are washed away, and the target protein is recovered by elution with imidazole (Figure 2). Polyhistidine- or HQ-tagged proteins can be purified under native and denaturing conditions. Additionally, HQ-tagged proteins may elute with a lower concentration of imidazole (50–100mM) compared to polyhistidine-tagged proteins.

The MagneHis™ Protein Purification System has the following features:

- **Simple:** No centrifugation or vacuum is required—lysis buffer is added directly to cells in culture medium.
- **Flexible:** MagneHis™ Ni-Particles are compatible with a variety of common buffers.
- **Quick:** No long incubations with lysozyme are required for cell lysis.
- **Efficient:** Binding capacity is up to 1mg of polyhistidine-tagged protein per 1ml of MagneHis™ Ni-Particles. Binding capacity of HQ-tagged proteins is comparable.
- **Versatile:** Perform purification manually or on an automated platform.
- **Convenient:** Complete system that includes all necessary reagents, including a unique lysis buffer and lyophilized DNase I.

Note: This product requires the use of a magnetic stand. Magnetic stands are sold separately.

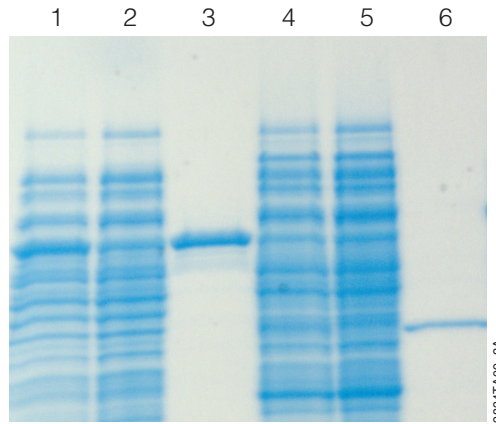


Figure 1. Purification of 6X polyhistidine-tagged fusion proteins using the MagneHis™ Protein Purification System. Lane 1, bacterial cell lysate expressing 6X polyhistidine-tagged firefly luciferase. Lane 2, column flowthrough of the lysate. Lane 3, eluted 6X polyhistidine-tagged firefly luciferase.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

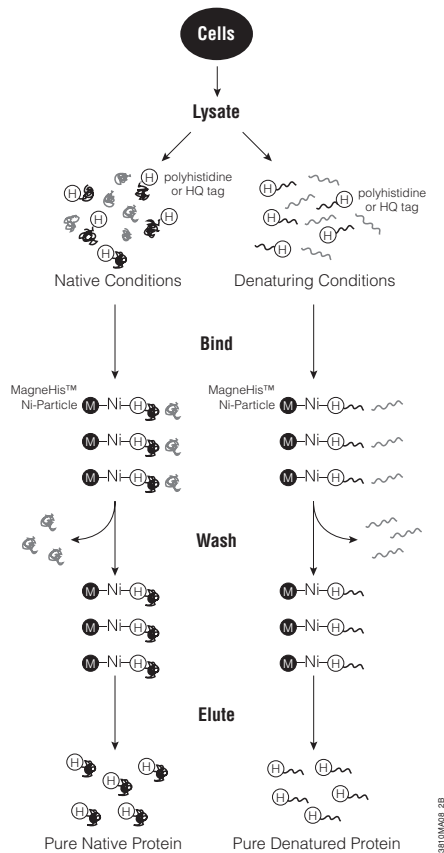


Figure 2. Diagram of the MagneHis™ Protein Purification System protocol.



2. Product Components and Storage Conditions

Product	Size	Cat.#
MagneHis™ Protein Purification System	65 reactions	V8500

Each system contains sufficient reagents for 65 manual purifications, each using 1ml of bacterial culture volume. This product requires the use of a magnetic stand (sold separately). Includes:

- 50ml MagneHis™ Binding/Wash Buffer
- 10ml MagneHis™ Elution Buffer
- 15ml FastBreak™ Cell Lysis Reagent, 10X
- 2ml MagneHis™ Ni-Particles
- 1 vial DNase I (lyophilized)

Product	Size	Cat.#
MagneHis™ Protein Purification System	325 reactions	V8550

Each system contains sufficient reagents for 325 manual purifications each using 1ml of bacterial culture volume. This product requires the use of a magnetic stand (sold separately). Includes:

- 250ml MagneHis™ Binding/Wash Buffer
- 50ml MagneHis™ Elution Buffer
- 60ml FastBreak™ Cell Lysis Reagent, 10X
- 10ml MagneHis™ Ni-Particles
- 2 vials DNase I (lyophilized)

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

This product requires the use of a magnetic stand.

Storage Conditions: Store all MagneHis™ Protein Purification System components at 4°C. **Do not freeze the MagneHis™ Ni-Particles.** The DNase I (lyophilized) may be stored at room temperature. Upon resuspension in water, store the DNase I in aliquots at -20°C. For convenience, the resuspended DNase I may be stored at 4°C for up to one week. **FastBreak™ Cell Lysis Reagent** may form a precipitate at low temperature. If this occurs, warm the reagent to room temperature before use.

3. MagneHis™ Protein Purification Protocol

Materials to be Supplied by the User

- 37°C incubator for flasks/tubes
- shaker
- magnetic separation stand to accommodate small-scale purifications using microcentrifuge tubes. Promega supplies Magnetic Separation Devices that can be used to hold 1–12 tubes. See Section 7.C for options.
- 1M imidazole solution (pH 8.0; for insect or mammalian cells, or culture medium)
- additional binding/wash buffer may be required if processing numerous insect cell, mammalian cell or culture medium samples
- solid NaCl or 5M NaCl solution

3.A. Preparation of Bacterial Cells

Bacterial cultures can be grown in tubes, flasks or 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 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.₆₀₀ < 6.0 for efficient processing.

3.B. Bacterial Cell Lysis

Two methods for lysis of bacterial cells using the FastBreak™ Cell Lysis Reagent, 10X, are presented in this section. In Method 1, cells are pelleted, and then the FastBreak™ Cell Lysis Reagent, 10X, diluted to 1X concentration, is added to the cell pellet with DNase I. Alternatively, FastBreak™ Cell Lysis Reagent, 10X, and DNase I are added directly to bacterial cultures as detailed in Method 2. This second method is quick and easy but may not be optimal for purification of some polyhistidine- or HQ-tagged proteins and will need to be tested for your specific fusion protein of interest. Sonication may also be used to prepare bacterial samples for protein purification; use 100µl of MagneHis™ Binding/Wash Buffer per milliliter of culture for cell pellet resuspension. Both lysis methods have been used successfully with Luria-Bertani and Terrific Broth medium.

Method 1: Lysis of Pelleted Bacterial Cells

See Section 3.A for preparation of cells before starting the experiment.

1. Determine the O.D.₆₀₀ for the fresh bacterial culture.
2. Centrifuge 1ml of bacterial culture at 10,000 × g for 2 minutes in a microcentrifuge. Remove the supernatant completely.
3. **Optional:** The cell pellet can be frozen at -20°C for 15 minutes or -70°C for 5 minutes. Some bacterial strains may require a freeze-thaw cycle to achieve maximal lysis.



4. For every 1 O.D.₆₀₀, dilute 10µl of FastBreak™ Cell Lysis Reagent, 10X, to 100µl (1X) by adding 90µl of NANOpure® or double-distilled water.
Do not process more than 1 O.D.₆₀₀ of culture per 100µl of 1X FastBreak™ Cell Lysis Reagent.
5. Resuspend the cell pellet in 1X FastBreak™ Cell Lysis Reagent.
Example: For a 3 O.D.₆₀₀ culture, use 300µl of 1X FastBreak™ Cell Lysis Reagent.
6. Resuspend lyophilized DNase I as indicated on the vial, and add 1µl to the lysed bacterial culture. Store the resuspended DNase I in aliquots at -20°C for long term or at 4°C for up to one week.
7. Incubate with shaking for 10-20 minutes at room temperature on a rotary mixer or shaking platform.

Method 2: Direct Lysis of Bacterial Cell Cultures

See Section 3.A for preparation of cells before starting the experiment.

1. Add 110µl of FastBreak™ Cell Lysis Reagent, 10X, (1/10 volume) directly to 1ml of fresh bacterial culture.
2. Resuspend lyophilized DNase I as indicated on the vial, and add 1µl per milliliter of original culture volume. Store the resuspended DNase I in aliquots at -20°C for long term or at 4°C for up to one week.
3. Incubate with shaking for 10-20 minutes at room temperature on a rotary mixer or shaking platform.

3.C. Purification of Polyhistidine- or HQ-Tagged Proteins from 1ml of Bacterial Culture Using MagneHis™ Ni-Particles

1. Add 500mM NaCl to HQ-tagged protein lysate (i.e., 0.03g NaCl per 1.0ml of lysate) to improve binding to MagneHis™ Ni-Particles.
2. Vortex the MagneHis™ Ni-Particles to a uniform suspension.
3. Add 30µl of MagneHis™ Ni-Particles either to cell pellet resuspended in 1X FastBreak™ Cell Lysis Reagent (from Section 3.B, Method 1, Step 7) or to 1.1ml of cell lysate (from Section 3.B, Method 2, Step 3).

Note: You may need to increase the amount of MagneHis™ Ni-Particles used for high-expressing proteins.

4. Invert tube to mix (approximately 10 times), and incubate for 2 minutes at room temperature. Make sure the MagneHis™ Ni-Particles are well mixed.
5. Place the tube in the appropriate magnetic stand for approximately 30 seconds to capture the MagneHis™ Ni-Particles. Using a pipette, carefully remove the supernatant.

6. Remove the tube from the magnetic stand. Add 150µl of MagneHis™ Binding/Wash Buffer to the MagneHis™ Ni-Particles and pipet to mix. If NaCl was added for binding, also use NaCl during washing. Make sure that particles are resuspended well.
7. Place the tube in the appropriate magnetic stand for approximately 30 seconds to capture the MagneHis™ Ni-Particles. Using a pipette, carefully remove the supernatant.
8. Repeat the wash step 2 times for a total of 3 washes.
9. Remove the tube from the magnetic stand. Add 100µl of MagneHis™ Elution Buffer, and pipet to mix.

Note: HQ-tagged proteins may elute at a lower concentration of imidazole (50–100mM) compared to polyhistidine-tagged proteins. The MagneHis™ Elution Buffer, which contains 500mM imidazole, can be diluted with MagneHis™ Binding/Wash Buffer or water, if required.

10. Incubate for 1–2 minutes at room temperature. Place in a magnetic stand to capture the MagneHis™ Ni-Particles. Using a pipette, remove the supernatant containing the purified protein. Analyze the samples by SDS-PAGE or by functional assay.

 See alternative elution conditions in Section 3.H.

Note: Steps 8–10 can be omitted and the sample containing the MagneHis™ Ni-Particles can be used directly for SDS-PAGE analysis. Add 30µl of 1X SDS gel-loading buffer to resuspend the MagneHis™ Ni-Particles. Load the sample directly onto a SDS-polyacrylamide gel.

3.D. Preparation of Insect or Mammalian Cells

Insect or mammalian cells can be cultured under normal conditions. Process cells at a cell density of 2×10^6 cells/ml of culture. Adherent cells may be removed from tissue culture plastic by scraping and resuspending in culture medium to this density. Cells may be processed in culture medium containing up to 10% serum. Processing more than the indicated number of cells per 1ml sample may result in reduced protein yield and increased nonspecific binding.

3.E. Lysis and Purification of Polyhistidine- or HQ-Tagged Proteins from 1ml Cultured Insect or Mammalian Cells

Note: This protocol is for purification of intracellularly expressed polyhistidine- or HQ-tagged proteins.

1. Add 110µl of FastBreak™ Cell Lysis Reagent, 10X, to 1ml of insect or mammalian cells in culture medium.

Note: We do not recommend adding 500mM NaCl to the FastBreak™ Cell Lysis Reagent, 10X, as it could result in particle clumping.



2. Resuspend lyophilized DNase I as indicated on the vial, and add 1 μ l to the lysed insect or mammalian cell culture. Store the resuspended DNase I in aliquots at -20°C for long term or at 4°C for up to one week.
3. Incubate with shaking for 10-20 minutes at room temperature on a rotary mixer or shaking platform.
4. Prepare MagneHis™ Binding/Wash Buffer by adding NaCl to a final concentration of 500mM (i.e., 0.03g NaCl per 1.0ml of MagneHis™ Binding/Wash Buffer).
Note: You may need to optimize the amount of NaCl added to the MagneHis™ Binding/Wash Buffer.
5. Vortex the MagneHis™ Ni-Particles to a uniform suspension.
6. Add 30 μ l of the MagneHis™ Ni-Particles to 1.1ml of cell lysate.
Note: You may need to optimize the volume of particles for purification. Add 1M imidazole (pH 8.0) to a final concentration of 20mM to decrease nonspecific binding of serum proteins (22 μ l of 1M imidazole per 1.1ml of sample).
7. Invert tube to mix (approximately 10 times), and incubate for 2 minutes at room temperature.
8. Place the tube in the appropriate magnetic stand for approximately 30 seconds to capture the MagneHis™ Ni-Particles. Using a pipette, carefully remove the supernatant.
9. Remove the tube from the magnetic stand. Add 500 μ l of MagneHis™ Binding/Wash Buffer containing 500mM NaCl to the MagneHis™ Ni-Particles and pipet to mix. Make sure that the particles are resuspended well.
10. Place the tube in the appropriate magnetic stand for approximately 30 seconds. Allow the MagneHis™ Ni-Particles to be captured, and carefully remove the supernatant using a pipette.
11. Repeat the wash step 2 times for a total of 3 washes.
12. Remove the tube from the magnetic stand. Add 100 μ l of MagneHis™ Elution Buffer and pipet to mix.
Note: HQ-tagged proteins may elute at a lower concentration of imidazole (50-100mM) compared to polyhistidine-tagged proteins. The MagneHis™ Elution Buffer, which contains 500mM imidazole, can be diluted with MagneHis™ Binding/Wash Buffer or water, if required.
13. Incubate for 1-2 minutes at room temperature. Place the tube in a magnetic stand to capture the MagneHis™ Ni-Particles with the magnet. Using a pipette, remove the supernatant containing the purified protein. Analyze the samples by SDS-PAGE or by functional assay.

3.F. Purification of Polyhistidine- or HQ-Tagged Proteins from 1ml Insect or Mammalian Cell Culture Medium

Note: This protocol is for purification of polyhistidine- and HQ-tagged proteins secreted into the culture medium. Cells should be removed from the medium before protein purification.

1. Vortex the MagneHis™ Ni-Particles to a uniform suspension.
2. Add 30µl of MagneHis™ Ni-Particles to 1.0ml of culture medium after removing cells.
Note: You may need to optimize the volume of particles used. Add 1M imidazole to a final concentration of 20mM to decrease nonspecific binding of serum proteins (20µl per 1.0ml sample). Adding 500mM NaCl (i.e., 0.03g NaCl per 1.0ml of medium) may improve HQ-tagged protein binding.
3. Invert tube to mix (approximately 10 times), and incubate for 2 minutes at room temperature.
4. Place the tube in the appropriate magnetic stand for approximately 30 seconds to capture the MagneHis™ Ni-Particles with the magnet. Using a pipette, carefully remove the supernatant.
5. Prepare MagneHis™ Binding/Wash Buffer by adding sodium chloride to a final concentration of 500mM (i.e., 0.03g NaCl per 1.0ml of MagneHis™ Binding/Wash Buffer).
6. Remove the tube from the magnet. Add 500µl of MagneHis™ Binding/Wash Buffer containing 500mM NaCl to the MagneHis™ Ni-Particles and pipet to mix. Make sure that the particles are resuspended well.
7. Place the tube in the appropriate magnetic stand for approximately 30 seconds to capture the MagneHis™ Ni-Particles with the magnet. Using a pipette, carefully remove the supernatant.
8. Repeat the wash step 2 times for a total of 3 washes.
9. Remove the tube from the magnet. Add 100µl of MagneHis™ Elution Buffer and pipet to mix.
Note: HQ-tagged proteins may elute with a lower concentration of imidazole (50-100mM) compared to polyhistidine-tagged proteins. The MagneHis™ Elution Buffer, which contains 500mM imidazole, can be diluted with MagneHis™ Binding/Wash Buffer or water.
10. Incubate for 1-2 minutes at room temperature. Place the tube in a magnetic stand to capture the MagneHis™ Ni-Particles. Using a pipette, remove the supernatant that contains the purified protein. Analyze the samples by SDS-PAGE or by functional assay.



3.G. Guidelines For Using Denaturing Conditions

Since the interaction of polyhistidine- or HQ-tagged fusion proteins and MagneHis™ Ni-Particles does not depend on tertiary structure of the tag, fusion proteins can be captured and purified using denaturing conditions. If the polyhistidine- or HQ-tagged fusion is expressed as inclusion bodies, solubilize by adding a strong denaturant such as 2-8M guanidine hydrochloride or urea.

This section should be used as a guideline for the purification of proteins expressed as inclusion bodies. Several parameters, including growth conditions, fusion tag, expression levels and solubilizing reagents, have a dramatic affect on overall yields of proteins purified from inclusion bodies. Conditions will need to be optimized for each individual protein. For additional information on the purification of fusion proteins, refer to references 2-7.

1. To determine if the polyhistidine- or HQ-tagged protein is located in inclusion bodies, perform the lysis step using FastBreak™ Cell Lysis Reagent, 10X, as described in Section 3.B, Method 2. Pellet the cellular debris by centrifugation and check the supernatant and the pellet for the presence of polyhistidine- or HQ-tagged protein by gel analysis. If the majority of the polyhistidine- or HQ-tagged protein is associated with the pellet, use denaturing conditions for isolation. Denaturants, such as guanidine or urea, can be added following lysis, but should not be mixed with the FastBreak™ Cell Lysis Reagent, 10X, prior to use.
2. Lyse the cell pellet in MagneHis™ Binding/Wash Buffer for Denaturing Conditions (Section 7.B) for 10–60 minutes. (1ml of culture should be resuspended in 200–600µl of MagneHis™ Binding/Wash Buffer for Denaturing Conditions). Proceed with purification using MagneHis™ Buffers for Denaturing Conditions (Section 7.B).
3. Denaturing conditions need to be used throughout the procedure; otherwise, the proteins may aggregate. We recommend making up denaturing buffers (Section 7.B). Solid guanidine-HCl or urea can be added directly to the MagneHis™ Binding/Wash and Elution Buffers. However, the addition of guanidine-HCl or urea will dilute the buffer, decreasing the imidazole concentrations; therefore, adding guanidine-HCl or urea directly to the MagneHis™ Binding/Wash and Elution Buffers provided may increase background and result in less eluted polyhistidine- or HQ-tagged protein.

3.H. 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 1).

Table 1. Alternative Elution Conditions.

Condition	Effect
Decrease MagneHis™ Elution Buffer.	Decreasing volume of Elution Buffer will concentrate protein (50µl Elution Buffer/30µl Ni-Particles can be used).
Perform second elution step.	In some cases, a second elution may release more polyhistidine- or HQ-tagged protein.
Use 0.5–1M imidazole.	Increasing imidazole concentration may elute more polyhistidine-tagged protein.
Use 0.1% trifluoroacetic acid (TFA) with or without acetonitrile.	Can be used to elute protein from the Ni-Particles and is useful in mass spectrometry analysis (see below).
Use 1M sodium citrate (pH 4–6).	Decreasing pH will elute protein. Neutralizing the eluate immediately after elution helps retain the function of the protein.
Dilute MagneHis™ Elution Buffer with water or Binding/Wash Buffer for desired imidazole concentration.	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) elution.

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 will be used for mass spectrometry analysis.

3.I. General Considerations

1. Polyhistidine proteins expressed for purification with this system should have at least five to six consecutive histidine residues located on the C- or N-terminus. The HQ tag contains three histidines and three glutamines (HQQHQ).
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. MagneHis™ Ni-Particles have been evaluated for compatibility with several common buffer components (Table 2).

Table 2. Compatibility of MagneHis™ Ni-Particles With Alternative Buffers.

Reagent	Concentration
cationic detergents	1%
glycerol	≤20%
guanidine-HCl	≤8M
NaCl	≤1M
Tris, MOPS, sodium phosphate, potassium phosphate	100mM
Triton® X-100	1%
Tween® 20	0.05%
urea	≤8M

5. 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.
6. 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 MagneHis™ Ni-Particles, include NaCl in the MagneHis™ Binding/Wash Buffer at a final concentration of 500mM NaCl. Lysozyme will produce a 12.5kDa band on a SDS-polyacrylamide gel.
7. Purified polyhistidine- or HQ-tagged protein can be quantitated using standard methods such as Bradford or BCA, but the imidazole in the MagneHis™ Elution Buffer may inhibit these assays. Either dialyze the sample or dilute to the optimal imidazole concentration for the protein quantitation reagent used (1:10 for BCA assay). Always include the same amount of imidazole in the standard curve as well.

8. 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).
9. We have used MagneHis™ Ni-Particles to purify polyhistidine- or HQ-tagged proteins generated in vitro by *E. coli* S30 lysate or wheat germ extract. Hemoglobin co-purifies with the polyhistidine- or HQ-tagged protein when isolating these proteins from rabbit reticulocyte-based in vitro translation systems; therefore, we recommend using the MagZ™ Protein Purification System. For additional details, contact Promega Technical Services.

4. Isolation of Polyhistidine- or HQ-Tagged Fusion Proteins on Automated Platforms

The manual protocol described in Section 3 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.

Specific instructions for use on several automated platforms are available at: www.promega.com/automethods/

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	Causes and Comments
Protein not expressed	Sequence or orientation not correct. Confirm the clone by sequencing.
	Cells not induced. Use the correct inducers, such as IPTG for T7-based expression in BL21(DE3) or JM109(DE3) cells.
	Expressed protein unstable. 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. Try different temperatures and times during induction (16–37°C).



5. Troubleshooting (continued)

<u>Symptoms</u>	<u>Causes and Comments</u>
Protein not expressed (continued)	Protein expressed as inclusion bodies. Check the lysate pellet or flowthrough for the presence of insoluble protein.
Protein isolated in low quantity or not eluting from the particles	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.
Polyhistidine- or HQ-tagged protein not binding to the particles	Adding 500mM NaCl to lysate may improve binding to the particles.
	Sequence incorrect. Confirm the clone by sequencing.
	Protein degradation. Add protease inhibitors.
	Media components interfere with binding. Centrifuge culture, remove media and resuspend pellet in 1X FastBreak™ Cell Lysis Reagent.
	Incorrect buffer pH. Problematic if trying to purify proteins secreted into the medium. Adjust pH to 7.5 before binding.
	Too few MagneHis™ Ni-Particles. Increase the amount of MagneHis™ Ni-Particles for binding.
Expressed protein disappears after purification	Protease contamination. Add protease inhibitors to the Lysis, Binding/Wash and Elution Buffers.
FastBreak™ Cell Lysis Reagent does not release the polyhistidine- or HQ-tagged 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 nonspecifically. Solubilize the protein with suitable detergent. If problem still exists, sonicate the sample.
A few proteins are co-eluted with the protein, even after extensive washing	Specific protein degradation. Add protease inhibitors to the Binding/Wash and Elution Buffers.
	Interacting protein in the host cell. Add 0.5-1M NaCl to washing step.

Symptoms	Causes and Comments
Eluted proteins form dimers or aggregates	Protein aggregation during denaturation. Addition of 0.5-1M NaCl may prevent nonspecific interaction. Denature samples at 70°C for 10 minutes, 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 1 or remove imidazole by dialysis or size-exclusion chromatography.
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. Specific protein degradation. Add protease inhibitors to the Binding/Wash and Elution Buffers.
After the binding step, Ni-Particles clump or cannot be fully magnetized	Nucleic acid contamination. Add DNase at 1µl/ml. 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.
Fusion proteins still present in the flowthrough	Not 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.
Purified protein dialysis	High imidazole. Elute using citrate (Table 1).



6. References

1. Betz, N.A. (2004) Efficient purification of His-tagged proteins from insect and mammalian cells. *Promega Notes* **87**, 29-32.
2. Carrio, M.M. and Villaverde, A. (2001) Protein aggregation as bacterial inclusion bodies is reversible. *FEBS Lett.* **489**, 29-33.
3. Carrio, M.M. and Villaverde, A. (2002) Construction and destruction of bacterial inclusion bodies. *J. Biotechnol.* **96**, 3-12.
4. Gu, Z. *et al.* (2002) Chromatographic methods for the isolation of, and refolding of proteins from, *Escherichia coli* inclusion bodies. *Protein Expr. Purif.* **25**, 174-9.
5. Sachdev, D. and Chirgwin, J.M. (1998) Solubility of proteins isolated from inclusion bodies is enhanced by fusion to maltose-binding protein or thioredoxin. *Protein Expr. Purif.* **12**, 122-32.
6. Mukhopadhyay, A. (1997) Inclusion bodies and purification of proteins in biologically active forms. *Adv. Biochem. Eng. Biotechnol.* **56**, 61-109.
7. Tsumoto, K. *et al.* (2003) Practical considerations in refolding proteins from inclusion bodies. *Protein Expr. Purif.* **28**, 1-8.

7. Appendix

7.A. O.D. Calculation

$O.D._{600} = 10 \times O.D._{600}$ of 1ml of a 1:10 dilution of the culture (diluted in medium) measured in a 1cm pathlength cuvette.

Note: Spectrophotometer should always be “zeroed” with a blank containing medium alone.

7.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

MagneHis™ Binding/Wash Buffer for Denaturing Conditions (pH 7.5)

- 100mM HEPES
- 10mM imidazole
- 2-8M guanidine-HCl or urea

MagneHis™ Elution Buffer for Denaturing Conditions (pH 7.5)

- 100mM HEPES
- 500mM imidazole
- 2-8M guanidine-HCl or urea

7.C. Related Products

Product	Size	Cat.#
HisLink™ Protein Purification Resin	50ml	V8821
HisLink™ 96 Protein Purification System	1 × 96 reactions	V3680
	5 × 96 reactions	V3681

Product	Size	Cat.#
MagneGST™ Protein Purification System	40 reactions	V8600
	200 reactions	V8603
MagneGST™ Glutathione Particles	4ml	V8611
	20ml	V8612
MagZ™ Protein Purification System	30 purifications	V8830

These products require the use of a magnetic stand.

Product	Size	Cat.#
JM109 Competent Cells, >10 ⁸ cfu/μg*	1ml	L2001
BL21(DE3)pLysS Competent Cells	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
RQ1 RNase-Free DNase*	1,000u	M6101
Guanidine-HCl, Molecular Grade	100g	H5381

*For Laboratory Use.

MagneSphere® Technology Magnetic Separation Stands

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	0.5ml	Z5331
	1.5ml	Z5332
	12 × 75mm	Z5333
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
	1.5ml	Z5342
	12 × 75mm	Z5343



7.C. Related Products (continued)

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[®] Vector Systems for cloning, refer to the *Flexi[®] Vector System Technical Manual #TM254* or visit: www.promega.com

^(a)U.S. Pat. Nos. 7,112,552 and 7,354,750.

^(b)Certain applications of this product may be covered by patents issued to parties other than Promega and applicable in certain countries. Because purchase of this product does not include a license to perform any of these patented applications, 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)This product is licensed for use under U.S. Pat. No. 6,174,704.

© 2002-2013 Promega Corporation. All Rights Reserved.

Flexi and MagneSphere are registered trademarks of Promega Corporation. FastBreak, HisLink, MagneGST, MagneHis and MagZ are trademarks of Promega Corporation.

NANOpure is a registered trademark of Barnstead/ThermoLyne Corporation. Speed Vac is a registered trademark of Savant Instruments, Inc. Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology Corporation. Tween is a registered trademark of ICI Americas, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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.