

PURIFYING HIS-TAGGED PROTEINS FROM INSECT AND MAMMALIAN CELLS

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The FastBreak™ Cell Lysis Reagent, 10X and the MagneHis™ Protein Purification System can be used to purify His-tagged proteins from insect and mammalian cell lysates and culture media in the presence or absence of serum. The FastBreak™ Reagent, 10X, is added directly to cells in culture, achieving gentle, efficient lysis in 15 minutes without centrifugation or mechanical disruption. Lysis can also be achieved by adding the FastBreak™ Reagent directly to attached or pelleted cells at a 1X concentration. The MagneHis™ Protein Purification System can be used to purify His-tagged proteins. The combination of the FastBreak™ Reagent and the MagneHis™ System allows efficient purification of His-tagged proteins from diverse sample types.

Introduction

Many different polypeptide fusion partners or affinity tags have been developed to facilitate purification of target proteins (1). The most commonly used tag for the purification and detection of recombinant expressed proteins is the His tag. Cloning vectors designed to generate His-tagged proteins contain 5–10 histidine residues at either the C- or N-terminus of the expressed protein. The His tag adds only 0.84kDa to the mass of the protein and is nonimmunogenic. Also, because the tertiary structure of the tag is not important for purification, His-tagged proteins can be purified using native or denaturing conditions. The affinity of histidine residues for immobilized nickel allows selective purification of His-tagged proteins (2,3). The MagneHis™ Ni-Particles^(a)

contained in the MagneHis™ Protein Purification System^(a) can bind up to 1mg of His-tagged protein per milliliter of particles (4), providing a fast, efficient method for purifying His-tagged proteins with high yield and low background in a highly scalable format.

The FastBreak™ Cell Lysis Reagent, 10X^(a,b) was developed to allow efficient lysis of *E. coli* cultures without the need for centrifugation or mechanical disruption. Following lysis, any released His-tagged protein can be purified directly using MagneHis™ Ni-Particles (5).

Bacterial expression of recombinant His-tagged proteins is a common technique. However, use of other systems, such as Sf9 insect cells, or HeLa or CHO mammalian cells, for expression of recombinant proteins either intracellularly or secreted into the culture medium is increasing. These eukaryotic expression systems may allow more natural processing and modification of recombinant His-tagged proteins (6–8).

In this report, we demonstrate use of the FastBreak™ Reagent and the MagneHis™ Protein Purification System with insect and mammalian cell lysates and culture media. An outline of the purification protocols from either culture medium or eukaryotic cells is depicted in Figure 1.

The FastBreak™ Cell Lysis Reagent and the MagneHis™ Protein Purification System provide an efficient method for purifying His-tagged proteins from insect and mammalian cell lysates and culture media.

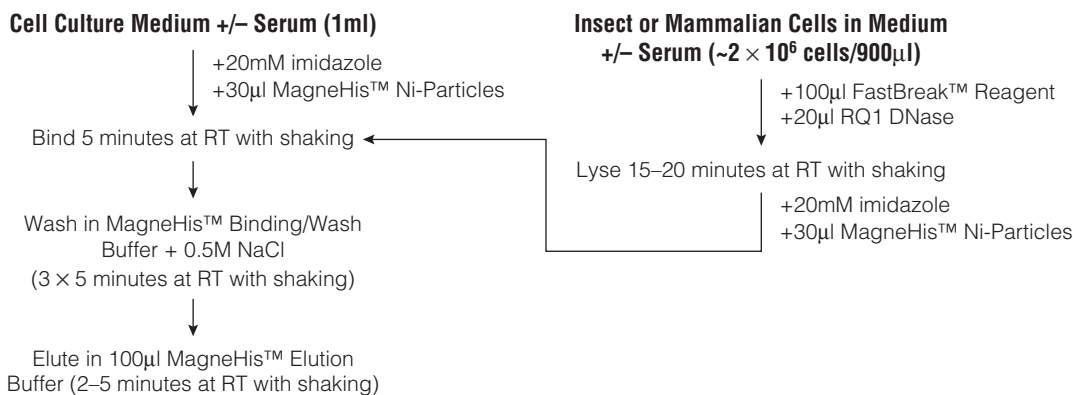


Figure 1. Flow diagram showing purification of His-tagged proteins from eukaryotic cell culture medium or lysates in the presence or absence of serum using the FastBreak™ Cell Lysis Reagent and the MagneHis™ Protein Purification System.

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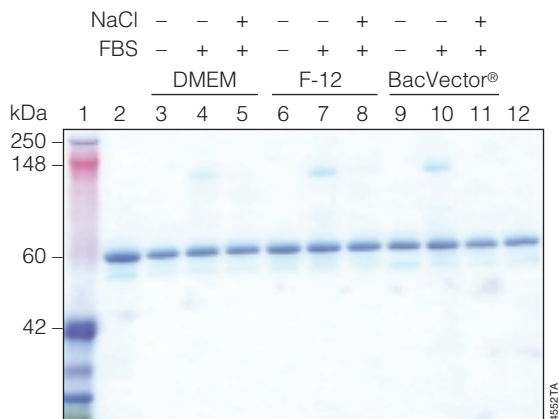


Figure 2. Purification of His-tagged thermostable luciferase from insect or mammalian cell culture media using the MagneHis™ Protein Purification System. Purified His-tagged thermostable luciferase (~5µg) was added to various culture media in the presence or absence of 10% fetal bovine serum (FBS) as indicated, then purified from 1ml of each medium using the MagneHis™ Protein Purification System according to the protocol provided in the supplied Technical Manual #TM060 (30µl particles/sample). Equivalent volumes (3µl) of each purified protein were analyzed on a 4–20% Tris-Glycine polyacrylamide gel (Invitrogen), followed by detection with SimplyBlue™ SafeStain (Invitrogen). Lane 1, prestained molecular weight marker (MultiMark®; Invitrogen). Lanes 2 and 12 contain an equivalent amount of the input luciferase protein for comparison. DMEM (Invitrogen), F-12 (Invitrogen), and BacVector® Insect Cell Medium (Novagen) were used as indicated. Lanes containing samples that were washed with MagneHis™ Binding/Wash Buffer containing 0.5M NaCl are indicated.

Compatibility of MagneHis™ Ni-Particles With Various Insect and Mammalian Cell Culture Media

To verify that the MagneHis™ Ni-Particles are compatible with purification of His-tagged proteins from commonly used insect and mammalian cell culture media, purified His-tagged thermostable luciferase (~5µg) was added to various culture media in the presence or absence of 10% fetal bovine serum. Culture media tested included Dulbecco's Modified Eagle Medium (DMEM), F-12 Medium and BacVector® Insect Cell Medium. The His-tagged luciferase was then purified from 1ml of medium using the MagneHis™ System according to the protocol in the supplied Technical Manual #TM060 (30µl particles/sample). For some samples containing serum, washes were performed using MagneHis™ Binding/Wash Buffer containing a final concentration of 0.5M NaCl to reduce nonspecific protein binding.

The MagneHis™ System was able to efficiently purify His-tagged thermostable luciferase from all culture media tested, either in the presence or absence of serum (Figure 2). Addition of 0.5M NaCl to the MagneHis™ Binding/Wash Buffer removed the few nonspecific proteins present in serum that bound to the particles (compare lanes 4 and 5, 7 and 8, and 10 and 11).

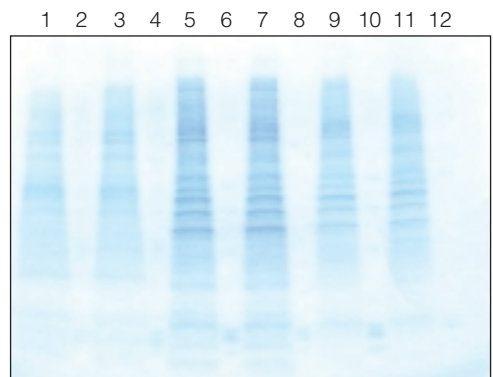


Figure 3. Effect of FastBreak™ Cell Lysis Reagent on lysis of insect and mammalian cells. Aliquots (900µl of ~2 × 10⁶ cells/ml) of Sf9 insect cells (lanes 1–4), HeLa cells (lanes 5–8), or CHO cells (lanes 9–12) were combined with 100µl 10X FastBreak™ Cell Lysis Reagent in the presence (lanes 3–4, 7–8, 11–12) or absence (lanes 1–2, 5–6, 9–10) of 20µl of RQ1 RNase-Free DNase. Pellet and supernatant fractions were collected by centrifugation (15 minutes at 14,000rpm in a microcentrifuge). Odd-numbered lanes contain supernatant fractions, and even-numbered lanes contain pellet fractions. Pellet fractions were resuspended in 1ml 1X Laemmli sample buffer. Equal volumes (15µl) of supernatant and pellet fractions were analyzed on a 4–20% Tris-Glycine polyacrylamide gel, followed by detection with SimplyBlue™ SafeStain (Invitrogen).

To investigate whether the presence of the FastBreak™ Reagent adversely affected purification, His-tagged luciferase was added to culture media containing FastBreak™ Reagent, then purified with the MagneHis™ System. The presence of FastBreak™ Reagent in the culture media neither enhanced nor reduced purification of the His-tagged luciferase (data not shown). Addition of the FastBreak™ Reagent to culture media may be advantageous for secreted proteins that are more hydrophobic and require the presence of detergents for efficient purification. In addition, verification that the culture medium is pH 7–7.5 should allow efficient purification of His-tagged proteins from other culture media as well.

Purifying His-Tagged Proteins Directly From Insect and Mammalian Cell Lysates Without Centrifugation

Sf9 insect cells (Novagen) and HeLa and CHO mammalian cells (ATCC) were cultured in the appropriate medium (DMEM, F-12, or BacVector® Insect Cell Medium). Aliquots (900µl) of each cell type were lysed by adding 100µl of FastBreak™ Reagent, 10X, in the presence or absence of RQ1 RNase-Free DNase (Cat.# M6101). Following incubation at room temperature for 20 minutes with shaking, supernatant and pellet fractions were analyzed as described in Figure 3. The FastBreak™ Reagent lysed both the insect and mammalian cells efficiently as seen by the lack of protein in pellet fractions compared with supernatant fractions (Figure 3). The presence of DNase did not alter lysis efficiency.

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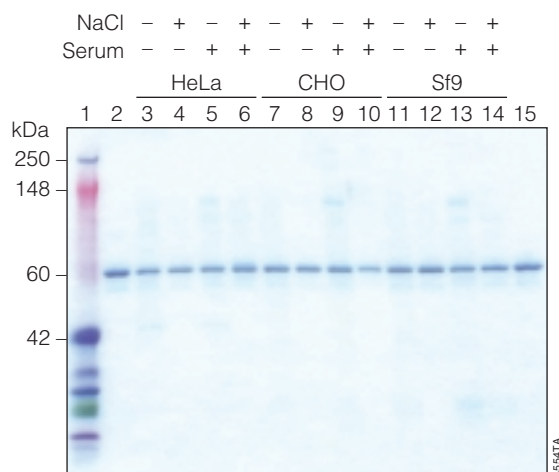


Figure 4. Purification of His-tagged thermostable luciferase using the FastBreak™ Cell Lysis Reagent and the MagneHis™ Protein Purification System. Cells were harvested by scraping to a final concentration of $\sim 2 \times 10^6$ cells/ml in culture medium, either in the presence or absence of serum as indicated. FastBreak™ Cell Lysis Reagent, 10X (100 μ l), $\sim 5\mu$ g of purified His-tagged thermostable luciferase, and 20 μ l RQ1 RNase-Free DNase were added to 900 μ l aliquots of each cell suspension. Samples were incubated for 15–20 minutes at room temperature. Following cell lysis, MagneHis™ Ni-Particles (30 μ l) and imidazole (20mM final concentration) were added and the particles incubated for 2–5 minutes at room temperature with shaking. After three 5-minute washes with 500 μ l MagneHis™ Binding/Wash Buffer and elution in 100 μ l MagneHis™ Elution Buffer, equivalent volumes (3 μ l) of purified protein were analyzed on a 4–20% Tris-Glycine polyacrylamide gel, then detected with SimplyBlue™ SafeStain (Invitrogen). Lane 1, prestained MultiMark® molecular weight marker (Invitrogen); lanes 2 and 15 contain an equivalent amount of the input luciferase protein for comparison; lanes 3–6, luciferase purified from HeLa cells in DMEM; lanes 7–10, CHO cells in F-12 medium; lanes 11–14, Sf9 cells in BacVector® Insect Cell Medium. Lanes containing samples that were washed with MagneHis™ Binding/Wash Buffer containing 0.5M NaCl are indicated.

To verify that lysates prepared using FastBreak™ Reagent were compatible with purification of His-tagged proteins, cells were harvested by scraping to a final concentration of $\sim 2 \times 10^6$ cells/ml in culture medium, either in the presence or absence of serum. One hundred microliters of FastBreak™ Reagent, 10X, and $\sim 5\mu$ g of purified His-tagged thermostable luciferase were added to 900 μ l aliquots of each cell suspension. RQ1 RNase-Free DNase (20 μ l) was also added to decrease sample viscosity and prevent MagneHis™ Ni-Particle clumping. The addition of RQ1 DNase was necessary for efficient purification (data not shown). Samples were incubated for 15–20 minutes at room temperature with shaking. Following cell lysis, MagneHis™ Ni-Particles (30 μ l) and imidazole (20mM final concentration) were added and the particles incubated for 2–5 minutes at room temperature with shaking. The addition of

imidazole during the binding step decreased nonspecific binding of proteins present in the lysates and serum (data not shown). The particles were then washed 3 times with 500 μ l of MagneHis™ Binding/Wash Buffer for 5 minutes with shaking at room temperature. These parameters may require further optimization for each particular target protein.

Bound proteins were eluted using the MagneHis™ Elution Buffer and analyzed by polyacrylamide gel electrophoresis. The results demonstrate that the combination of the FastBreak™ Reagent and the MagneHis™ Protein System efficiently purified His-tagged luciferase from the insect and mammalian cell lysates (Figure 4). The addition of 0.5M NaCl (final concentration) to the wash buffer removed background caused by nonspecific protein binding.

Purifying His-Tagged Proteins From Harvested Insect or Mammalian Cell Lysates

In addition to purifying His-tagged luciferase from cell lysates made by adding FastBreak™ Reagent, 10X directly to cells in culture media, cells were also harvested by centrifugation and the cell pellets resuspended in 1X FastBreak™ Cell Lysis Reagent (diluted in water). Efficient purification of His-tagged luciferase was also observed from these samples (data not shown). This allows the user the flexibility to remove the culture medium if desired. The addition of DNase during the lysis step, and imidazole during the binding step, is highly recommended, as well as the addition of 0.5M NaCl to the wash buffer.

Conclusions

The FastBreak™ Cell Lysis Reagent, 10X and the MagneHis™ Protein Purification System can be used together to efficiently and cleanly purify His-tagged proteins from insect and mammalian cell lysates. The MagneHis™ Protein Purification System can also be used to purify His-tagged proteins from common tissue culture media for both insect and mammalian cells in the presence or absence of serum. Only minor modifications to the standard protocol for bacterial cultures are required for purification from these diverse sources. All experiments were performed with His-tagged protein added directly to culture media or lysates. However, the conditions described in this article can be used as guidelines for His-tagged expressed proteins.

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References

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Protocols

MagneHis™ Protein Purification System Technical Manual
#TM060, Promega Corporation.
(www.promega.com/tbs/tm060/tm060.html)

FastBreak™ Cell Lysis Reagent, 10X, Product Information
#9PIV857, Promega Corporation.
(www.promega.com/tbs/9piv857/9piv857.html)

Ordering Information

Product	Size	Cat.#
FastBreak™ Cell Lysis Reagent, 10X ^(a,b)	10ml	V8571
	40ml	V8572
	100ml	V8573
MagneHis™ Protein Purification System ^(a)	65 reactions	V8500
	325 reactions	V8550
RQ1 RNase-Free DNase*	1,000u	M6101

*For Laboratory Use.

^(a)Patent Pending.

^(b)This product is licensed for use under U.S. Pat. No. 6,174,704.

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