Taking the Spin Out of Cell Lysis

FastBreak™ Cell Lysis Reagent For Protein Purification

By Jude Stevens, B.S., and Gary Kobs, B.S., Promega Corporation

Abstract

FastBreak™ Cell Lysis Reagent, 10X is a new cell lysis buffer for use with protein purification systems. The FastBreak™ Reagent can be added directly to E. coli *cultures for gentle, efficient lysis in 10 to 15 minutes without the need for centrifugation or mechanical disruption. We demonstrate the compatibility of the FastBreak™ Buffer with the MagneHis™ Protein Purification System in both manual and robotic formats.*

The FastBreak™ Cell Lysis Reagent is designed for the efficient, gentle lysis of *E. coli* cultures without the need for centrifugation or mechanical cell disruption.

Introduction

One of the most common methods used in the field of proteomic research is the purification of recombinant proteins expressed in *E. coli*. This process involves culturing cells under optimal conditions, followed by a concentration or harvesting step (usually centrifugation) and either mechanical or chemical cell lysis. When using a few samples this process is satisfactory. However, as the need for high-throughput protein purification increases, techniques limiting the number of samples that can be handled efficiently are not adequate.

To meet the need for a lysis method compatible with high-throughput processing, we developed the FastBreak™ Cell Lysis Reagent[a,b,c]. This reagent is designed for efficient, gentle lysis of *E. coli* cultures without the need for centrifugation or mechanical cell disruption. The reagent is provided as a 10X concentrate and contains a proprietary nonionic detergent to facilitate lysis. The reagent is added directly to *E. coli* culture, the cells are disrupted and protein is released during a brief incubation. Recombinant proteins can be directly screened in the resulting extract or purified by the addition of an appropriate affinity matrix (i.e., Promega MagneHis™ Protein Purification System[b,c] [Cat.# V8500]). This format allows the procedure to be performed manually or on a robotic platform, such as the Beckman Coulter Biomek® 2000 or FX workstations, allowing for high-throughput applications.

Purification of His-Tag Fusion Protein: Manual and Robotic Formats

We evaluated the compatibility of the FastBreak™ Cell Lysis Reagent with protein purification systems using the MagneHis™ Protein Purification System in both manual and automated formats. Cell lysate preparation was identical for the two methods. A 61kDa His-tagged protein was expressed in BL21(DE3) Star™ cells. Cell cultures were grown at 25°C in LB with ampicillin. His-tagged protein expression was induced using 1mM IPTG in cultures at an $O.D._{600}$ of between 0.4 and 0.6. Cultures were grown for an additional 16 hours after induction. Cells were lysed by adding 100µl of FastBreak™ Cell Lysis Reagent to 900µl of induced culture and incubating for 10 minutes at room temperature with shaking.

His-tagged protein was purified manually as described in the MagneHis™ Protein Purification System Technical Manual (#TM060). Figure 1 illustrates that the FastBreak™ Reagent efficiently lyses *E. coli* and does not interfere with subsequent protein purification.

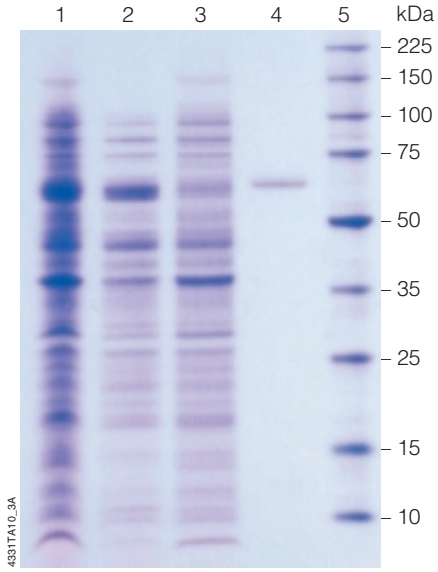


Figure 1. Manual purification of a 6X His-tagged fusion protein using FastBreak™ Cell Lysis Reagent and the MagneHis™ Protein Purification System. Lane 1, bacterial cell lysate expressing 6X His-tagged firefly luciferase lysed by the addition of FastBreak™ Reagent; lane 2, postlysis cell supernatant demonstrating that the protein is solubilized; lane 3, flowthrough of cell lysate after the addition of MagneHis™ Ni-Particles; lane 4, eluted 6X His-tagged firefly luciferase; lane 5, Broad Range Protein Molecular Weight Markers (Cat.# V8491).

Figure 2 shows the uniformity of cell lysis and purification using a robotic format. His-tagged protein was purified in a 96-well format using the MagneHis™ System and the Beckman Coulter Biomek® FX. A downloadable method is available from Promega at: www.promega.com/automethods/

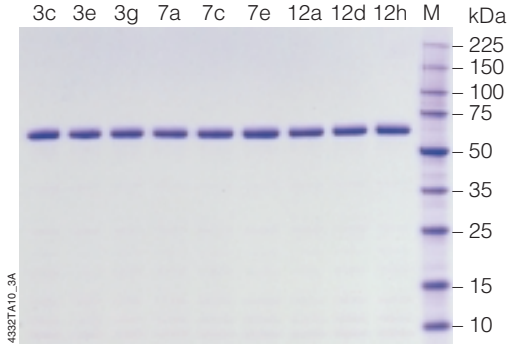


Figure 2. Reproducibility of the automated procedure. Lanes denote the well number of a 96-well plate from which the sample cultures were lysed using FastBreak™ Cell Lysis Reagent and purified using the MagneHis™ Protein Purification System on a Biomek® FX workstation. Lane M, Broad Range Protein Molecular Weight Markers (Cat.# V8491).

Optimizing Cell Lysis/Purification

The addition of secondary reagents may further enhance the lysis and subsequent purification when cultures are grown under certain conditions or using certain *E. coli* strains as hosts. When lysing cultures that have reached a high density (e.g., $O.D._{600} > 5$) or when using the *E. coli* strain, BL21(DE3)pLysS, the lysates produced are often viscous due to the release of genomic DNA. This can be resolved by adding DNase and incubating for 10–20 minutes at room temperature on a shaker or rotary platform. The lysate will become less viscous after DNase treatment.

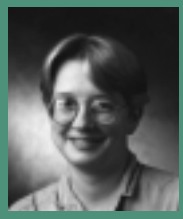
Lysozyme (from egg white) can also be used to enhance cell lysis and is active in the presence of FastBreak™ Reagent. To use lysozyme, prepare a fresh 20mg/ml solution of lysozyme in TE buffer (pH 7.4). Add 10µl of lysozyme solution per ml of lysate and incubate for 10 minutes. Adding DNase is recommended after lysis treatment to reduce the viscosity of the lysate. Since lysozyme binds to MagneHis™ Ni-Particles[b], add NaCl (0.5M final concentration) to the lysate following the DNase treatment when using the MagneHis™ Protein Purification System. This will disrupt lysozyme binding but will not affect binding of the His-tagged protein to the MagneHis™ Particles.

Conclusion

The FastBreak™ Cell Lysis Reagent offers a convenient format for the in-media lysis of *E. coli* cells expressing recombinant proteins. In addition to efficient cell lysis, the composition of the FastBreak™ Reagent does not interfere with downstream protein purification using the MagneHis™ System.

Protocols

- *FastBreak™ Cell Lysis Reagent, 10X, Product Information Sheet* #9PIV857, Promega Corporation. (www.promega.com/tbs/9piv857/9piv857.html)
- *MagneHis™ Protein Purification System Technical Manual* #TM060, Promega Corporation. (www.promega.com/tbs/tm060/tm060.html)



Jude Stevens, B.S.
Senior Process Development Scientist

Gary Kobs, B.S.
Product Manager

Ordering Information

Product	Size	Cat.#
FastBreak™ Cell Lysis Reagent, 10X[a,b,c]	10ml	V8571
	40ml	V8572
	100ml	V8573
MagneHis™ Protein Purification System[b,c]	65 reactions	V8500
	325 reactions	V8550

[a]This product is licensed for use under U.S. Pat. No. 6,174,704.

[b]Patent pending.

[c]Certain applications of this product 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.