Certificate of Analysis

FastBreak™ Cell Lysis Reagent, 10X:

Part# 9PIV857

Revised 12/21

Part No.	Size (ml)
V857A	15
V857B	60
V857C	100

Description: FastBreak™ Cell Lysis Reagent[a,b] is a proprietary formulation of buffer and detergents designed to lyse *E. coli* cells in culture.

Storage Conditions: Store at 4–25°C.

Usage Note: A precipitate may form at low temperature. If this occurs, warm the reagent to room temperature before use.

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Quality Control Assays

Lysis Efficiency

Cells are induced to express luciferase and then grown in LB overnight to an $OD_{600} > 3$. One hundred microliters (100µl) of FastBreak™ Reagent is added to 900µl of cell culture and mixed for 10 minutes at room temperature. A sample of the crude lysate is removed and centrifuged to remove cell debris. Luciferase activity in the supernatant of the cleared lysate must be at least 50% of the luciferase activity measured in the the same volume of crude lysate.

Promega

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Part# 9PIV857
Printed in USA. Revised 12/21.

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

[b]Patent Pending.

Usage Information

1. Description

FastBreak™ Cell Lysis Reagent is designed for the efficient, gentle lysis of *E. coli* cultures without the need for centrifugation or mechanical cell disruption. This reagent is provided as a 10X concentrate and contains a proprietary nonionic detergent to facilitate lysis. The reagent is added directly to *E. coli* cultures. FastBreak™ Reagent has a different formulation than the other cell lysis buffers used in Promega protein purification systems, which are designed for use with cell pellets. Following a brief incubation, the cells are disrupted, and the protein of interest is released. Recombinant proteins can be directly screened in the resulting extract or purified by the addition of an appropriate affinity matrix such as the MagneHis™ Protein Purification System (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, for high-throughput applications.

2. Manual Protocol

A. Cell Lysis

Reagents to Be Supplied by the User

- *E. coli* culture
- shaker or rotary platform

1. Add 100µl of FastBreak™ Reagent to 900µl of bacterial culture (see Note 1).
2. Mix for 10–15 minutes at room temperature on a shaker or rotary platform. Adequate mixing is necessary to ensure complete lysis.
3. Proceed with screening or purification. We have observed reduced yields when using the FastBreak™ Buffer with the MagneGST™ Protein Purification System (Cat.# V8600).

Notes:

1. When lysing cultures that have reached a high density (e.g., $OD_{600} > 5$) 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 (e.g., 20–40 units of RQ1 DNase [Cat.# M6101] or 4 Kunitz units of Sigma DNase [Cat.# D5025] freshly resuspended in 10X FastBreak™ Reagent) and incubating for 10–20 minutes at room temperature on a shaker or rotary platform. The lysate will become less viscous after DNase treatment. If the lysate is still too viscous for easy handling, add more DNase or increase the incubation time.
2. When using MagneHis™ Ni-Particles, it may not be apparent that DNase is needed until after the particles have been added to the cell lysate. In very viscous lysates, the particles will not mix uniformly in the lysate and may not be captured by the magnet. The samples may be treated with DNase after addition of the particles.
3. Lysozyme can be used to enhance cell lysis. Lysozyme (from egg white) is active in the presence of FastBreak™ Reagent. Freshly prepare a 20mg/ml solution of lysozyme in TE buffer (pH 7.4). Add 10µl of lysozyme solution per milliliter of lysate and incubate for 10 minutes. Due to the increased cell lysis when using lysozyme, it is also necessary to add DNase to reduce the viscosity of the lysate. Since lysozyme binds to MagneHis™ Ni-Particles, NaCl (0.5M final concentration) should be added to the lysate following the DNase treatment when using the MagneHis™ Protein Purification System.
4. FastBreak™ Reagent can be used to lyse cells that have been stored at 4°C overnight. However, storage of cells may not be optimal for all expressed proteins.
5. In some cases a precipitate may be seen. This occurs most often with Terrific Broth purification steps.

FastBreak™ Cell Lysis Reagent is a component of the MagneHis™ Protein Purification System. For a detailed protocol using the FastBreak™ Cell Lysis Reagent with the MagneHis™ Protein Purification System, please see the *MagneHis™ Protein Purification System Technical Manual #*TM060:
(**www.promega.com/resources/protocols/technical-manuals/0/magnehis-protein-purification-system-protocol/**)

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