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PeriPreps™ Periplasting Kit

Cat. No. PS81100

The PeriPreps™ Periplasting Kit facilitates the release of proteins contained within the periplasmic space of *E. coli* cells by combining digestion of the cell wall using Ready-Lyse™ Lysozyme with osmotic shock.[1-3] Ready-Lyse Lysozyme is a non-avian, non-mammalian lysozyme that is stable at –20°C. Directing the export of recombinant proteins to the periplasmic space has advantages over the expression of proteins in the cytoplasm.[3] First, export to the periplasm may improve the solubility and activity of expressed proteins. Second, purification of proteins exported to the periplasm will likely be easier because fewer contaminating proteins are present in this fraction versus whole cell lysates.

The PeriPreps Kit is also useful for the release of proteins comprising the remaining spheroplast fraction. Addition of OmniCleave™ Endonuclease to the lysis reaction substantially improves handling and recovery of recombinant proteins by decreasing the viscosity of cell lysates caused by nucleic acids. The enzyme degrades single- and double-stranded DNA and RNA to oligonucleotides of 2-4 bases.

Product Specifications

Storage: Store the Omnicleave™ Endonuclease at -20°C. Store the remainder of the kit at 4°C.

Note: *Diluted OmniCleave (in PeriPreps Lysis Buffer) is stable for up to 1 year at 4°C.*

Storage Buffer: OmniCleave Endonuclease is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton® X-100, and 1 mM dithiothreitol.

Desc.	Concentration	Quantity
PeriPreps™ Periplasting Kit Contents		
The PeriPreps Periplasting Kit contains reagents sufficient to lyse 100, 1-ml bacterial cultures or 5 grams of cells.		
PeriPreps™ Periplasting Buffer		5 ml
PeriPreps™ Lysis Buffer		10 ml
OmniCleave™ Endonuclease 4,000 U	@ 200 U/µl	20 µl
1 M $MgCl_2$ Solution		200 µl
0.5 M EDTA		400 µl
Purified Water		5 ml

PeriPreps Periplasting Buffer: 200 mM Tris-HCl (pH 7.5), 20% sucrose, 1 mM EDTA, and 30 U/µl Ready-Lyse Lysozyme.

PeriPreps Lysis Buffer: 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, and 0.1% deoxycholate.

Quality Control: The PeriPreps PeriPlasting Kit is function-tested by purifying, quantitating, and assaying the total protein content of both the periplastic and spheroplastic fractions of an *E. coli* culture expressing a recombinant assayable protein.

Related Products: The following products are also available:

– Ready-Lyse™ Lysozyme Solution

– OmniCleave™ Endonuclease

Contaminating Activity Assays: All components of the PeriPreps Periplasting Kit are free of detectable endogenous exo- and endonuclease and protease activities, with the exception of the inherent nuclease activity of the Omnicleave Endonuclease component.

General Considerations

1. **Cell Culture Conditions:** The PeriPreps Kit was developed for *E. coli* cells. The reagents will likely work with other Gram-negative bacteria, although we have not yet tested other strains. We recommend growing bacterial cells to late log phase only, as older cell cultures in stationary phase commonly demonstrate some resistance to lysozyme treatment. If the expression of recombinant protein is excessive, cells may prematurely lyse.[3] Therefore, for most cell cultures, we recommend against the use of rich medium (e.g., Terrific Broth[4]) or higher growth temperatures that might induce excessive protein synthesis.[3] Elevated growth temperatures may also induce the synthesis of heat shock proteins that may cause aggregation of recombinant protein with endogenous host proteins.
2. **Inclusion of Nuclease During Cell Lysis:** OmniCleave Endonuclease is added to PeriPreps Lysis Buffer at the discretion of the user. Inclusion of a nuclease will generally improve the yield of protein and the ease of handling of the lysates. The user may decide, however, that addition of a nuclease is undesirable in some cases. For example, the use of a nuclease should be avoided if residual nuclease activity or transient exposure to the magnesium cofactor will interfere with subsequent assays or uses of the purified protein. The addition of EDTA to the lysate to inactivate OmniCleave Endonuclease, likewise, may interfere with subsequent assay or use of the purified protein.
3. **Effect of Temperature on Lysis:** Ready-Lyse Lysozyme is optimally active at room temperature. Lysis at lower temperatures (e.g., 0°C-4°C) requires additional incubation time; we recommend extending incubation times 2- to 4-fold.
4. **Inactivation of Added Nuclease:** Chelation of the magnesium cofactor through the addition of EDTA to the lysate inactivates OmniCleave Endonuclease.
5. **Stability of Diluted Nuclease:** Diluted OmniCleave Endonuclease (as described in the protocols) is stable in PeriPreps Lysis Buffer for up to one year if stored at 4°C.

6. **Checking for the Release of Periplasmic Proteins:** We recommend analyzing an aliquot of both the periplasmic and spheroplastic fractions by SDS-PAGE (two microliters of each fraction is generally sufficient for visualization by Coomassie Brilliant Blue). The presence of unique proteins or the enrichment of specific proteins in each fraction indicates successful fractionation. For example, if the strain of *E. coli* contains a high-copy number plasmid with the ampicillin resistance marker, then the presence of β-lactamase (31.5 kDa) mainly in the periplasmic fraction indicates successful fractionation. Other *E. coli* proteins found in the periplasmic space include alkaline phosphatase (50 kDa) and elongation factor Tu (43 kDa).
7. **Subsequent Processing of Lysates Treated with Nucleases:** Following hydrolysis of nucleic acids with OmniCleave Endonuclease, lysates may contain substantial amounts of mono- or oligonucleotides. The presence of these degradation products may affect further processing of the lysate. For example, nucleotides may decrease the binding capacity of anion exchange resins by interacting with the resin.

Protocol for the Preparation of Periplasmic and Spheroplastic Proteins from a 1 ml Bacterial Culture

One milliliter (up to 2 OD_{600}) of a fresh bacterial cell culture may be processed as described below. If the OD_{600} of the culture is different from 2 or the volume of culture is greater than 1 ml, adjust the volumes of the reagents proportionately. The addition of OmniCleave Endonuclease will facilitate the purification of target proteins; nevertheless, depending on the goals of the experiment, the nuclease and magnesium cofactor may be omitted (See General Considerations).

1. Grow the bacterial cell culture and induce expression accordingly. The cells should be in log phase or early stationary phase (See General Considerations).
2. Pellet 1 ml of cell culture by centrifugation in a microcentrifuge.

   Note: *For best results, the cells must be freshly prepared.*
3. To degrade contaminating nucleic acids, dilute 2 µl of OmniCleave Endonuclease up to 1 ml with PeriPreps Lysis Buffer for each milliliter of Lysis Buffer needed in Step 10 (For the stability of diluted OmniCleave Endonuclease, see General Considerations).
4. Thoroughly resuspend the cells in 50 µl of PeriPreps Periplasting Buffer either by vortex mixing or by pipeting until the cell suspension is homogeneous.

   Note: *Excessive agitation may cause premature lysing of the spheroplasts resulting in contamination of the periplasmic fraction with cytoplasmic proteins.*
5. Incubate for 5 minutes at room temperature.
6. Add 50 µl of purified water at 4°C and mix by inversion.
7. Incubate for 5 minutes on ice.
8. Pellet the lysed cells by centrifugation in a microcentrifuge for 2 minutes at room temperature.
9. Transfer the supernatant containing the periplasmic fraction to a clean tube.
10. Resuspend the pellet in 100 µl of PeriPreps Lysis Buffer.

11. If OmniCleave Endonuclease was added to the PeriPreps Lysis Buffer in Step 3, add 1 µl of 1.0 M $MgCl_2$ and mix by inversion.
12. Incubate for 5 minutes at room temperature (See General Considerations).
13. Pellet cellular debris by centrifugation in a microcentrifuge for 5 minutes at room temperature.
14. Transfer the supernatant containing the spheroplastic fraction to a clean tube.
15. If OmniCleave Endonuclease was added to the PeriPreps Lysis Buffer in Step 3, add 2 µl of 500 mM EDTA to chelate the magnesium (The final concentration of EDTA in the lysate is 10 mM).

Protocol for the Preparation of Total Cellular Protein from a 1 ml Bacterial Culture

One milliliter (up to 2 OD_{600}) of a fresh bacterial cell culture may be processed as described below. If the OD_{600} of the culture is different from 2 or the volume of culture is greater than 1 ml, adjust the volumes of the reagents proportionately. The addition of OmniCleave Endonuclease will facilitate the purification of target proteins; nevertheless, depending on the goals of the experiment, the nuclease and magnesium cofactor may be omitted (See General Considerations).

1. Pellet 1 ml of cells by centrifugation in a microcentrifuge for 2 minutes at room temperature. The cells can either be previously frozen or freshly prepared (1 ml of *E. coli* cells [at 10^9 cells/ml] will generally yield 100-150 µg of total protein).
2. To degrade contaminating nucleic acids, dilute 2 µl of OmniCleave Endonuclease up to 1 ml with PeriPreps Lysis Buffer for each milliliter of Lysis Buffer needed in Step 5 (For the stability of diluted OmniCleave Endonuclease, see General Considerations).
3. Thoroughly resuspend the cells in 50 µl of PeriPreps Periplasting Buffer either by vigorously vortex mixing or by pipeting until the cell suspension is homogeneous.
4. Incubate for 5 minutes at room temperature.
5. Add 50 µl of PeriPreps Lysis Buffer.
6. If OmniCleave Endonuclease was added to the PeriPreps Lysis Buffer in Step 2, add 1 µl of 1.0 M $MgCl_2$ and mix by inversion.
7. Incubate for 5 minutes at room temperature (See General Considerations).
8. Pellet cellular debris by centrifugation in a microcentrifuge for 5 minutes at room temperature.
9. Transfer the supernatant containing the cellular protein fraction to a clean tube.
10. If OmniCleave Endonuclease was added to the PeriPreps Lysis Buffer in Step 5, add 2 µl of 500 mM EDTA to chelate the magnesium (The final concentration of EDTA in the lysate is 10 mM).

Protocol for the Preparation of Periplasmic and Spheroplastic Proteins from >1 ml Bacterial Culture

The amount of reagents required for this protocol is determined by the wet weight of the cell pellet. The addition of OmniCleave Endonuclease will facilitate the purification of target proteins; nevertheless, depending on the goals of the experiment, the nuclease and magnesium cofactor may be omitted (See General Considerations).

1. Grow the bacterial cell culture and induce expression accordingly. The cells should be in log phase or early stationary phase (See General Considerations).
2. Pellet the cell culture by centrifugation at a minimum of 1,000 x g for 10 minutes at room temperature.

   Note: *The cells must be fresh, not frozen. Determine the wet weight of the cell pellet.*
3. To degrade contaminating nucleic acids, dilute 2 µl of OmniCleave Endonuclease and 10 µl of 1.0 M $MgCl_2$ up to 1 ml with PeriPreps Lysis Buffer for each milliliter of Lysis Buffer needed in Step 10 (For the stability of diluted OmniCleave Endonuclease, see General Considerations).
4. Thoroughly resuspend the cells in a minimum of 2 ml of PeriPreps Periplasting Buffer for each gram of cells either by vortex mixing or by pipeting until the cell suspension is homogeneous.

   Note: *Excessive agitation may cause premature lysing of the spheroplasts resulting in contamination of the periplasmic fraction with cytoplasmic proteins.*
5. Incubate for 5 minutes at room temperature.
6. Add 3 ml of purified water at 4°C for each gram of original cell pellet weight and mix by inversion.
7. Incubate for 10 minutes on ice.
8. Pellet the lysed cells by centrifugation at a minimum of 4,000 x g for 15 minutes at room temperature.
9. Transfer the supernatant containing the periplasmic fraction to a clean tube.
10. Resuspend the pellet in 5 ml of PeriPreps Lysis Buffer for each gram of original cell pellet weight.
11. Incubate at room temperature for 10 minutes (If included, OmniCleave Endonuclease activity will result in a significant decrease in viscosity; continue the incubation until the cellular suspension has the consistency of water).
12. Pellet cellular debris by centrifugation at a minimum of 4,000 x g for 15 minutes at 4°C.
13. Transfer the supernatant containing the spheroplast fraction to a clean tube.
14. If OmniCleave Endonuclease was added to the PeriPreps Lysis Buffer, add 20 µl of 500 mM EDTA for each milliliter of the resultant spheroplastic fraction, to chelate the magnesium (The final concentration of EDTA in the lysate is 10 mM).

Protocol for the Preparation of Total Cellular Protein from >1 ml Bacterial Culture

The amount of reagents required for this protocol is determined by the wet weight of the cell pellet. The addition of OmniCleave Endonuclease will facilitate the purification of target proteins; nevertheless, depending on the goals of the experiment, the nuclease and magnesium cofactor may be omitted (See General Considerations).

1. Pellet the cell culture by centrifugation at a minimum of 1,000 x g for 10 minutes at room temperature. The cells can either be previously frozen or freshly prepared. Determine the wet weight of the cell pellet.
2. To degrade contaminating nucleic acids, dilute 2 µl of OmniCleave Endonuclease and 10 µl of 1.0 M $MgCl_2$ up to 1 ml with PeriPreps Lysis Buffer for each milliliter of Lysis Buffer needed in Step 5 (For the stability of diluted OmniCleave Endonuclease, see General Considerations).
3. Thoroughly resuspend the cells in a minimum of 2 ml of PeriPreps Periplasting Buffer for each gram of cells either by vortex mixing or by pipeting until the cell suspension is homogeneous.
4. Incubate 15 minutes at room temperature (See General Considerations).
5. Add 3 ml of PeriPreps Lysis Buffer for each gram of original cell pellet weight and mix by inversion.
6. Incubate at room temperature for 10 minutes (If included, OmniCleave Endonuclease activity will result in a significant decrease in viscosity; continue the incubation until the cellular suspension has the consistency of water).
7. Pellet cellular debris by centrifugation at a minimum of 4,000 x g for 15 minutes at 4°C.
8. Transfer the supernatant containing the spheroplast fraction to a clean tube.
9. If OmniCleave Endonuclease was added to the PeriPreps Lysis Buffer, add 20 µl of 500 mM EDTA for each milliliter of the resultant spheroplastic fraction, to chelate the magnesium (The final concentration of EDTA in the lysate is 10 mM).

Troubleshooting Cellular Fractionation

Cells lysed prematurely (periplasmic fraction is viscous)

1) **Expression of recombinant protein is excessive.** Grow cells in less nutritive broth or at a lower temperature.
2) **Resuspension of cells is too harsh.** Grow cells until late log phase and ensure that cells are completely, but gently resuspended in PeriPreps Periplasting Buffer.
3) **Cells are unusually susceptible to lysis.** Omit osmotic shock (Steps 6 and 7, pages 3 and 5). To isolate spheroplast proteins, resuspend cells in either an amount of purified water equivalent to the amount of PeriPreps Periplasting Buffer used in Step 4, or by the addition of PeriPreps Lysis Buffer in Step 10. Continue with the protocols as outlined.

Cells did not lyse

1) **Digestion of bacterial cell walls is incomplete.** Ensure that cells are completely resuspended in PeriPreps Periplasting Buffer. Increase the time of incubation following the addition of Periplasting Buffer and purified water. Alternatively, the cells were harvested in stationary phase instead of late-log phase, and consequently became resistant to lysozyme treatment.

Recombinant protein is not in the periplasmic fraction

1) **Cells did not lyse.** See above.
2) **Recombinant protein is either not expressed or not exported.** Check for expression by preparing a total cellular protein extract and analyzing the resultant extract by SDS-PAGE. If the protein is expressed, analyze the periplasmic and spheroplastic fractions by SDS-PAGE analysis to determine if native periplasmic proteins are found predominately in the periplasmic fraction. If native periplasmic proteins partition correctly, then either export of the recombinant protein failed, or the recombinant protein is not exportable to the periplasm.

References:

1. Schoenfeld, T. (1998) *Epicentre Forum* **5** (1), 5.
2. Carter, P. *et al.*, (1992) *Biotechnology* **10**, 163.
3. French, C. *et al.*, (1996) *Enzyme and Microbiol. Technol.* **19**, 332.
4. Tartof, K.D. and Hobbs, C.A. (1987) *Focus* **9** (2), 12.

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