

Lyse-Aid™ Recombinant Lysozyme



Cat. Nos. R1003E & R1004E

DESCRIPTION

Lyse-Aid Recombinant Lysozyme is a highly pure and stable enzyme used for efficient lysis of gram negative bacteria such as *E. coli*. The enzyme attacks peptidoglycans in bacterial cell walls and hydrolyzes the glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine. Lyse-Aid has a specific activity of 1,700 KU/mg, which is 250 times greater than that of chicken egg white lysozyme, so less enzyme is required for *E. coli* lysis. With optimal activity at neutral pH (6.0 – 8.0), Lyse-Aid is compatible with common buffers as well as Semba's protein extraction reagents. Small amounts of Lyse-Aid (3-5 KU/gram of cell paste) enhance the efficiency of protein extraction with SembaSonic™ Protein Extraction Reagent. In the absence of detergents, lysis of *E. coli* can be achieved by treatment of frozen-and-thawed cells with 45-60 KU/gram. Lyse-Aid may be substituted for chicken egg white lysozyme in most cell lysis protocols at a ratio of 10 KU Lyse-Aid per milligram chicken egg white lysozyme.

LYSIS OF E. COLI BY FREEZE-THAW PLUS LYSE-AID TREATMENT

The freeze-thaw step ruptures the cell membrane allowing the Lyse-Aid enzyme access to the cell wall. In the recommended protocol below, pelleted cells are first resuspended in buffer before freezing.

1. Collect cells by centrifugation at 9,000 x g for 15 min in a tared tube or bottle. Remove as much supernatant as possible and determine the weight (mass) of the pellet.
2. Thoroughly resuspend the cell pellet in lysis buffer (50 mM Tris-Cl, 50 mM NaCl, pH 7.5) using 7 ml per gram of cell paste and mix by pipetting up and down. If required, EDTA or other protease inhibitors can be added to the buffer. Generally a ratio of 100 µl lysis buffer/ml of the original culture volume will give good results.
3. Freeze the resuspended cells completely at -20°C or lower temperature. Rapid freezing can be accomplished using a dry ice-ethanol bath.
4. Completely thaw the frozen cell suspension at room temperature, mixing to ensure that the solution is homogeneous.
5. Add 7.5 KU Lyse-Aid per ml lysis buffer (45-60 KU per gram original cell paste). **IMPORTANT:** Do not add Lyse-Aid until a uniform cell suspension has been obtained. If the enzyme is added prematurely, partial lysis may result.
6. *Optional:* add 25 units of Benzonase® Nuclease (Cat. Nos. R1005E – R1010E) per ml lysis buffer used for resuspension. Benzonase will degrade all nucleic acids and reduce the viscosity of the extract.

7. Incubate the mixture on a shaking platform or rotating mixer at a slow setting for 20 min at room temperature. Incubation at lower temperatures will slow the enzymatic lysis and necessitate a longer reaction. Incubation above 40°C is not recommended.
8. Centrifuge at 16,000 x g for 20 min at 4°C. Shorter centrifugation times and lower g forces may be used as long as lysate clarification is adequate.
9. Transfer the supernatant, which contains the extracted soluble proteins, to a fresh tube. The pellet can be further processed to purify inclusion bodies.

LYSIS OF E. COLI WITH LYSE-AID AND SEMBASONIC™ PROTEIN EXTRACTION REAGENT

Lyse-Aid is ideal for use in combination with SembaSonic Protein Extraction Reagent (Cat. Nos. R1011E, R1012E) and Benzonase (Cat. Nos. R1009E, R1010E) for convenient lysis of *E. coli* without the need for mechanical disruption. The SembaSonic reagent contains a non-ionic detergent that normally does not interfere with protein activity and enhances the efficiency of cell lysis. For each gram of cell paste prepared as in Step 1, resuspend in 5 ml 1X SembaSonic reagent containing 25 mM Tris-Cl, pH 8.0, add 5 KU Lyse-Aid, and incubate 10-20 minutes at room temperature. If desired, add 125 U Benzonase to degrade nucleic acids and reduce viscosity of the protein extract. Another option for convenience is to use SembaSonic Master Mix (Cat. Nos. R1016E, R1017E), which is a pre-mixed combination of all three of the reagents: Benzonase, Lyse-Aid, and SembaSonic Protein Extraction Reagent in 25 mM Tris buffer, pH 8.0.

STORAGE

Store at -20°C. Lyse-Aid Recombinant Lysozyme is supplied in 50 mM Tris-Cl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, and 50% glycerol, pH 7.5. The preparation is stable for 2 years when stored as directed. The enzyme can be diluted with 50 mM Tris-Cl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, pH 7.5. Diluted samples can be stored at 4°C for several days without loss of activity.

UNIT DEFINITION

One unit of Lyse-Aid Recombinant Lysozyme is defined as the amount of enzyme that causes a decrease of 0.025 A₄₅₀ per minute at 37°C in a 1.0 mg/ml suspension of *E. coli* cells in 0.5X SembaSonic Protein Extraction Reagent diluted with 50 mM Tris-Cl, pH 7.5.

Note 1 KU = 1000 units.

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