Ready-Lyse™ Lysozyme Solution

Cat. Nos. R1802M, R1804M, and R1810M
1. Introduction

Ready-Lyse™ Lysozyme Solution is a stabilized lysozyme preparation for the lysis of Gram-negative bacteria such as *E. coli*, as well as some Gram-positive bacteria. It is supplied as a ready-to-use solution, in quantities of 2, 4, or $10 \times 10^6$ units, that is stable at $-20^\circ$C, and retains activity with repeated use. Ready-Lyse Lysozyme Solution is also more active than egg-white lysozyme, the traditional enzyme used for bacterial lysis, and is optimally active at the neutral pH values common to most lysis buffers. Egg-white lysozyme is optimally active at pH values >9. In the pH 6.5-7.5 range, the specific activity of Ready-Lyse Lysozyme Solution is approximately 200 times higher than that of egg-white lysozyme.

As less Ready-Lyse Lysozyme Solution is needed to lyse a given amount of bacteria, losses due to nonspecific binding are virtually eliminated in nucleic acid purifications. In contrast, egg-white lysozyme can bind to and precipitate DNA, RNA, or negatively charged proteins, reducing yield. For example, in Fig. 1, nearly 50% of the DNA in a plasmid purification has coprecipitated with the egg-white lysozyme (lane 7). An equivalent amount (in activity units) of Ready-Lyse Lysozyme Solution causes much less precipitation of DNA (compare lane 6 to lane 7).

2. Product Specifications

**Storage:** Store only at $-20^\circ$C in a freezer without a defrost cycle.

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

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**Figure 1. Decreased loss of DNA with Ready-Lyse™ Lysozyme Solution compared to egg-white lysozyme.** pH79 cosmid DNA (500 µg/ml) was incubated for 15 minutes at 22°C with either 5 µg (30 KU)/ml of Ready-Lyse Lysozyme (RL), 500 µg/ml of egg-white lysozyme (EW), or no lysozyme (C) in conditions typically used for lysis of *E. coli* (25 mM Tris [pH 8.0], 10 mM EDTA). The solutions were then microcentrifuged for 10 minutes. The supernatants were removed and the pellets were resuspended in TE buffer containing 0.1% SDS. Supernatants (lanes 1-3) and pellets (lanes 5-7) were then analyzed by electrophoresis in a 0.8% agarose gel.
Unit Definition: One unit produces a decrease in $A_{450}$ of 0.001 per minute at 25°C with a suspension (0.5 mg/ml) of lyophilized E. coli K802 cells in 50 mM Tris-HCl (pH 7.5).

Contaminating Activity Assays: Ready-Lyse Lysozyme Solution is free of detectable exonuclease and endonuclease activities.

3. Protocols for Using Ready-Lyse Lysozyme Solution

These protocols are offered as guidelines for the use of Ready-Lyse Lysozyme and can be scaled, depending on the particular application. The precise amount of enzyme needed for complete digestion may vary with different strains of E. coli (see Notes).

Protocol for Preparing Mini-Lysates with Ready-Lyse Lysozyme
1. Grow a culture of E. coli to $A_{600} = 1.9$.
2. Divide the culture into 1.5-ml aliquots.
3. Pellet the cells by centrifugation.
4. Completely resuspend the cells in 25 µl of TES Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, and 100 mM NaCl).
5. Dilute Ready-Lyse Lysozyme to a concentration of 250 U/µl in TES Buffer.
6. Add 1 µl of the diluted enzyme to each aliquot of resuspended cells and mix.
7. Incubate at room temperature with occasional swirling.

Protocol for Preparing Large-Scale Lysates with Ready-Lyse Lysozyme
1. Grow a 1,000-ml culture of E. coli to $A_{600} = 1.9$.
2. Pellet the cells by centrifugation.
3. Completely resuspend the cells on ice in 25 ml of TES Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, and 100 mM NaCl).
4. Add 250,000 U of undiluted Ready-Lyse Lysozyme and swirl gently.
5. Incubate at room temperature or in a water bath at 25°C.

Notes

Lysis: Lysis occurs quite rapidly at room temperature, but is greatly slowed by cold temperatures. With either protocol, complete digestion should occur within 15 minutes at room temperature; lysis is indicated by a gradual clearing of the culture with a concomitant increase in viscosity. Following lysis, the lysate can be treated according to standard protocols for the purification of nucleic acids or proteins.

Bacterial Strains: Ready-Lyse Lysozyme will digest the cell walls of most Gram-negative bacteria. For Gram-positive strains, adjust the concentration of Ready-Lyse Lysozyme to 5X that suggested in the above protocols. Addition of greater than 5X the concentration of Ready-Lyse Lysozyme is unlikely to result in lysis.
4. Related Products

The following products are also available:

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**GELase™ Agarose Gel-Digesting Preparation**

**Plasmid-Safe™ ATP-Dependent DNase**

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**PeriPreps™ Periplasting Kit**

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