Manual

Ready-Lyse Lysozyme Solution

For Research Use Only. Not for use in diagnostic procedures.

Ready-Lyse™ Lysozyme Solution is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

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1. Introduction

Ready-Lyse Lysozyme Solution is a stabilised 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 4 or 10 × 10^6 units, that is stable at -20 °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).

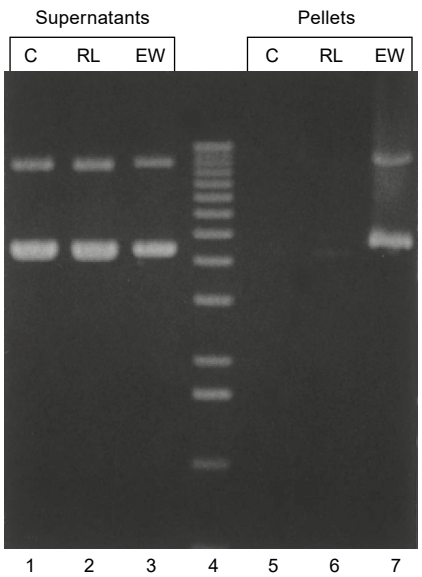

Figure 1. Decreased loss of DNA with Ready-Lyse Lysozyme Solution compared to egg-white lysozyme. pHC79 cosmid DNA (500 µg/mL) was incubated for 15 minutes at 22 °C with either 5 µg (30 KU)/mL of Ready-Lyse Lysozyme (RL), 500 µg/mL of egg-white lysozyme (EW) or no lysozyme (C) in conditions typically 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 analysed by electrophoresis in a 0.8% agarose gel.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part number	Volume
Ready-Lyse Lysozyme Solution	4,000,000 U	R1804M	Ready-Lyse Lysozyme Solution (~30,000 U/µL*)	E0057-D2	Varies*
	10,000,000 U	R1810M	Ready-Lyse Lysozyme Solution (~30,000 U/µL*)	E0057-D3	Varies*

*Unit concentration value (U/µL) and the corresponding tube volume appears on the tube label as each lot varies.

3. Product specifications

Storage: Store only at -20 °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.

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 lyophilised *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.

4. 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).

4.A. 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.

4.B. 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.

5. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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