

# DNA Purification from Hard-to-Lyse Bacteria Using Ready-Lyse™ Lysozyme and the MasterPure™ DNA Purification Kit

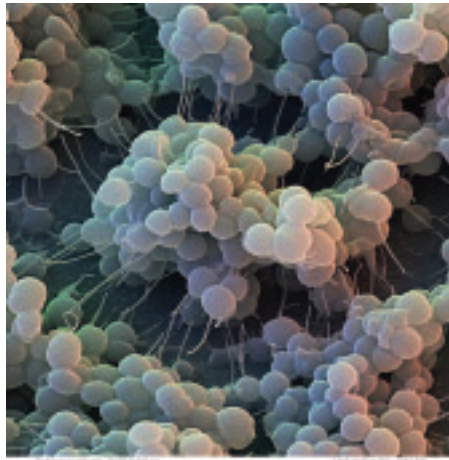
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Lysis of *E. coli* and other gram-negative bacteria, followed by DNA purification with the MasterPure™ DNA Purification Kit, is straight-forward, simple, and provides high yields of DNA. Lysing gram-positive bacteria, however, frequently requires the use of Ready-Lyse™ Lysozyme and a few additional steps. Ready-Lyse Lysozyme is a non-mammalian, non-avian, recombinant lysozyme with the following advantages over egg white lysozyme:

- Unlike egg white lysozyme, Ready-Lyse Lysozyme is not positively charged at neutral pH and does not bind negatively-charged molecules, such as DNA.<sup>1</sup>
- The specific activity of Ready-Lyse Lysozyme is 200-fold higher than egg white lysozyme, so less exogenous protein is added to the sample.
- Ready-Lyse Lysozyme is stable in solution at -20°C, which allows long-term storage and eliminates the need to prepare fresh enzyme solution from powder for each use.
- Ready-Lyse Lysozyme lyses both gram-negative and gram-positive bacteria.

Ready-Lyse Lysozyme has been used to lyse a variety of gram-positive bacteria that are normally resistant to lysis using many standard DNA purification procedures. EPICENTRE developed the following protocol for lysis of gram-positive bacteria before extracting DNA with the MasterPure DNA Purification Kit or the MasterPure™ Complete DNA and RNA Purification Kit.

1. Pellet, by centrifugation, 1 to 1.5 ml of an overnight, gram-positive bacterial



Scanning electron micrograph of *Staphylococcus aureus* by David Scharf. [www.microscopy-today.com/Scharf.html](http://www.microscopy-today.com/Scharf.html)

2. culture and discard the supernatant.
2. Add 150 µl of T<sub>10</sub>E<sub>1</sub> (10 mM Tris-HCl, pH 7.5; 1 mM EDTA) and vortex to resuspend the cell pellet.
3. Add 1 µl of Ready-Lyse Lysozyme to each resuspended pellet of bacteria (from 1 to 1.5 ml of culture).
4. Incubate at 37°C for 30 minutes to overnight. (Note: Tested *Streptococcus* and *Listeria* bacteria required overnight lysis. See Table 1)
5. Dilute 1 µl of Proteinase K (50 µg/µl) into 150 µl of 2X T&C Lysis Solution (both are provided in the MasterPure DNA Purification Kit, or sold separately) for each 1 to 1.5 ml of culture pelleted.
6. Add 150 µl of the Proteinase K/Lysis solution to the sample and mix thoroughly.

7. Incubate at 65°C to 70°C for 15 minutes, briefly vortexing every 5 minutes.
8. Cool the samples to 37°C.
9. Add 1 µl of RNase A (5 µg/µl, provided in the kit, or sold separately) to each sample and mix thoroughly.
10. Incubate at 37°C for 30 minutes.
11. Place the samples on ice for 3 to 5 minutes, and proceed with Part B of the MasterPure™ DNA Purification Kit Protocol.

Using the above procedure, we have tested five representative gram-positive bacterial species to demonstrate the effectiveness of Ready-Lyse Lysozyme, the required lysis times, and the DNA yields (Table 1).

As shown in Table 1, not all gram-positive bacteria require Ready-Lyse Lysozyme for cell lysis. After growth in brain-heart infusion broth, *Staphylococcus*, for example, lyses in the presence of the 2X T&C Lysis Solution alone. In contrast, the *Listeria* and *Streptococcus* species tested required an overnight incubation with the Ready-Lyse Lysozyme. In all cases, the EPICENTRE's MasterPure DNA Purification Kit provides sufficient bacterial DNA to perform downstream techniques such as hybridization, restriction enzyme digestion, direct genomic DNA sequencing, fosmid library production, or PCR amplification.

## Reference

1. EPICENTRE Forum (1994) 1(4), 4.

[www.epicentre.com/readylyse.asp](http://www.epicentre.com/readylyse.asp)

### Ready-Lyse™ Lysozyme Solution

R1802M	2 x 10 <sup>6</sup> U
R1804M	4 x 10 <sup>6</sup> U
R1810M	10 x 10 <sup>6</sup> U

### MasterPure™ DNA Purification Kit (for isolating TNA or DNA)

MCD85201 200 Purifications

#### Contents:

Red Cell Lysis Solution  
Tissue and Cell Lysis Solution  
MPC Protein Precipitation Reagent  
2X T&C Lysis Solution  
TE Buffer  
RNase A  
Proteinase K

Species	Culture Medium	Ready-Lyse™ Incubation Time	DNA Yield µg/ml Culture
<i>Bacillus subtilis</i>	Brain-Heart Infusion	30 min.	4.3
<i>Listeria monocytogenes</i>	Brain-Heart Infusion	Overnight	3.3
<i>Staphylococcus aureus</i>	Brain-Heart Infusion	Does not require Ready-Lyse	8
<i>Staphylococcus epidermidis</i>	Brain-Heart Infusion	Does not require Ready-Lyse	4.3
<i>Streptococcus mutans</i>	Todd-Hewitt	Overnight	3.0

Table 1. Growth culture medium, lysis times, and DNA yield for representative species of gram-positive bacteria using Ready-Lyse™ Lysozyme and the MasterPure™ DNA Purification Kit.