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MasterPure™ Gram Positive DNA Purification Kit

Cat. Nos. MGP04020 and MGP04100

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1. Introduction

The MasterPure™ Gram Positive DNA Purification Kit provides all of the reagents needed to purify DNA from gram positive bacteria. These bacteria lyse more readily after treatment with Ready-Lyse™ Lysozyme and the Gram Positive Cell Lysis Solution. Ready-Lyse Lysozyme is a stable solution of a non-mammalian, non-avian recombinant lysozyme, with high specific activity and no net charge at neutral pH. Thus, there is no waiting to dissolve the lysozyme and it does not bind DNA.[1,2]

2. Product Specifications

Storage: Store the Proteinase K, Ready-Lyse Lysozyme and RNase A at –20°C in a freezer without a defrost cycle. Store the remainder of the kit at room temperature for ease of use.

Storage Buffers: RNase A is supplied in a 50% glycerol solution containing 25 mM ammonium acetate (pH 4.6); Ready-Lyse Lysozyme is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1% Triton® X-100; Proteinase K is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10 mM $CaCl_2$, 0.1% Triton® X-100, and 1 mM DTT.

Quality Control: The MasterPure Gram Positive DNA Purification Kit is function-tested by purifying DNA from *Bacillus subtilis*. DNA quality and yield are assayed by agarose gel electrophoresis, spectrophotometry, and use as a template for PCR.

3. Kit Contents

Desc.	Concentration	Quantity
The MasterPure™ Gram Positive DNA Purification Kit is available in 20- and 100-purification sizes. The 100-purification kit contains:		
Gram Positive Cell Lysis Solution		15 ml
MPC Protein Precipitation Reagent		20 ml
Ready-Lyse™ Lysozyme		100 µl
RNase A	@ 5 µg/µl	100 µl
Proteinase K	@ 50 µg/µl	100 µl
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)		20 ml

4. Related Products

The following products are also available:

– Ready-Lyse™ Lysozyme

5. Gram Positive DNA Purification Protocol

1. Pellet by centrifugation, 1.0 ml of an overnight gram positive bacterial culture. Discard the supernatant.
2. Add 150 µl of TE Buffer and vortex to resuspend the cell pellet.
3. Add 1 µl of Ready-Lyse Lysozyme to each resuspended pellet (from 1.0 ml culture) of bacteria.
4. Incubate at 37°C for 30 minutes to overnight. See examples in Table 1.
5. Dilute 1 µl of Proteinase K (50 µg/µl) into 150 µl of Gram Positive Lysis Solution for each 1.0 ml of culture pellet.
6. Add 150 µl of the Proteinase K/Gram Positive Lysis Solution to the sample and mix thoroughly.
7. Incubate at 65-70°C for 15 minutes, vortexing briefly every 5 minutes.
8. Cool the samples to 37°C.
9. Place the samples on ice for 3-5 minutes and then proceed with DNA Precipitation.

Table 1. Examples of DNA Yields from Gram Positive Bacterial Species.

Bacterial Species	Culture Medium	Ready-Lyse Incubation Time	DNA Yield µg/ml
Bacillus subtilis	Brain-Heart Infusion (BHI)	30 minutes	9.0
Listeria monocytogenes	BHI	Overnight	3.3
Staphylococcus aureus	BHI	Not needed	8.0
Staphylococcus epidermidis	BHI	Not needed	4.3
Streptococcus mutans	Todd-Hewitt	Overnight*	3.0

**Addition of mutanolysin (not included) can shorten the time to ~30 minutes*

DNA Precipitation

1. Add 175 µl of MPC Protein Precipitation Reagent to 300 µl of lysed sample and vortex mix vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at >10,000 x g in a microcentrifuge.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 1 µl of RNase A (5 µg/µl) to each sample and mix thoroughly.
5. Incubate at 37°C for 30 minutes.
6. Add 500 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
7. Pellet the DNA by centrifugation at 4°C for 10 minutes at >10,000 x g in a microcentrifuge.
8. Use a pipet tip to remove the isopropanol without dislodging the DNA pellet.
9. Rinse the pellet with 70% ethanol. Centrifuge briefly if the pellet is dislodged.
10. Resuspend the DNA in 35 µl of TE Buffer.

6. References:

1. Hoffman, L.M. and Jarvis B.W. (2003) *Epicentre Forum* **10** (3), 3.
2. Jarvis, B.W. and Hoffman L.M. (2004) *Epicentre Forum* **11** (3), 5.

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