Manual

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MasterPure Gram Positive DNA Purification Kit

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MasterPure™ Gram Positive DNA Purification Kit is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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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 and Gram-negative bacteria. Gram-positive 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.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part number	Volume
MasterPure Gram Positive DNA Purification Kit	100 purifications	MGP04100	Ready-Lyse Lysozyme Solution (~ 30,000 U/µL)	E0057-D1	100 µL
			TE Buffer	SS000001-D3	20 mL
			Proteinase K (50 µg/µL)	SS000099-D2	100 µL
			RNase A (5 µg/µL)	SS000213-D2	100 µL
			MPC Protein Precipitation Reagent	SS000399-D2	20 mL
			MasterPure Gram Positive Cell Lysis Solution (2X)	SS000402-D4	15 mL

3. 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 (Rohm & Haas); 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.

4. 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.

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 or streptolysin (not included) can shorten the time to ~30 minutes

Table 1. Examples of DNA yields from gram positive bacterial species.

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 × 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 pipette 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.

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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