MPure-12

MPure Bacterial DNA Extraction Kit

Revision Date: 2019-12
Cat. No. 117022600 / 48 tests

Trade Name and Intended Use

MPure Bacterial DNA Extraction Kit is used with the MPure-12 Nucleic Acid Purification System for extraction and purification of genomic DNA from both gram-positive and gram-negative bacteria.

Application

Nucleic acids extracted and purified from MPure Bacterial DNA Extraction Kit can be used in a number of downstream applications, including PCR, qPCR, Sequencing (NGS), Microarray, RFLP and Southern Blot Analysis.

Description of Symbols Used

The following are graphical symbols used in or found on MP Biomedicals' products and packaging. They are explained in more detail in the European Standard BS EN ISO 15223-1:2012.

Use by

Temperature Limitation

LOT Batch Code
Synonyms:
Lot Number, Batch Number

Kit Components

Kit Contents *(for 48 extractions)*	Quantity
Reagent Cartridge	48 pcs (4 x 6 x 2)
Reaction Chamber	48 pcs (4 x 6 x 2)
Tip Holder	48 pcs (24 x 2)
Filtered Tip	50 pcs (25 x 2)
Piercing Pin	50 pcs (25 x 2)
Sample Tube (2 mL)	50 pcs (25 x 2)
Elute Tube (1.5 mL)	50 pcs (25 x 2)
BL2 Buffer	1 pc (25 mL)
Barcode Paper	1 pc

Reagent Cartridge Content

well-1: Proteinase K solution (40 µL)
well-2: Lysis Buffer 2 (720 µL)
well-3: Binding Buffer 1 (720 µL)
well-4: Magnetic Bead Solution (800 µL)
well-5: Washing Buffer 1 (1000 µL)
well-6: Washing Buffer 2 (1000 µL)
well-7: Washing Buffer 3 (1000 µL)
well-8: Elution Buffer (1000 µL)
well-9: Elution Buffer (1000 µL)
well-10: empty

Storage

Store at room temperature (15–25°C). Do not freeze the reagent cartridges. The kits are stable for 12 months under these conditions. Store the purified DNA at 4°C (short-term) or aliquot and store at -70°C (long-term) before performing the downstream analysis.

Starting Material

Bacterial pellet/colony from culture, cell-free body fluids, liquid transport media, urine and environmental samples (water, soil, etc.). If using paraffin-embedded tissue sections as samples, we recommend extracting DNA with the MPure FFPE DNA Extraction Kit. If using tissue as samples, we recommended using the MPure Tissue DNA Extraction Kit. The types and amounts of starting material for use in MPure Bacterial DNA purification procedures are shown in the table below:

Sample Type	Target Nucleic Acid	Sample Volume (Amount of starting material)	Elution Volume
Bacteria pellet	Genomic DNA	200-400 µL /Up to 10^9 bacteria (OD_{600} ~ 3)	50-300 µL
Bacterial colony		200-400 µL /1-3 colony	
Tissue		200-400 µL /1-30 mg	
Urine		200-400 µL /5-50 mL	
Cell-free body fluids		200-400 µL	
Liquid transport media		200-400 µL	
NOTE: Before extraction, adjust sample volume with BL2 Buffer			

Sample Preparation

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling. The buffer BL2 (supplied in the kit) is specialized for bacterial cell wall lysis and can be used to resuspend the bacterial pellet before extraction. For *mycobacterium spp.* (e.g. MTB), use buffer BL3 for bacterial cell wall lysis (BL3 buffer is supplied in the MPure TB DNA Extraction Kit (Cat. No. 117022800)

The table below describes recommendations for processing the samples prior to nucleic acid extraction:

Sample type	Procedure
For viscous samples e.g. BAL (Broncho-Alveolar Lavage), sputum or other mucous specimen	**Recommended pretreatment: Liquefaction** 1. Prepare a fresh DTT stock solution for liquefaction* (IM DTT stock solution is equal to 15%) 2. Adjust the final DTT concentration in the sample to 0.15% (1:100 dilution of the stock solution) 3. Incubate the sample (e.g., with shaking at 850 rpm for 30 min at 37°C) until it is easy to pipette the sample. 4. Pellet bacteria by centrifugation at 14,000 x g for 10 min 5. Discard supernatant and resuspend the pellet in 220 µL Buffer BL2 6. Transfer 200 µL of suspension to the sample tube (supplied in the kit) *The liquefaction of the sample can also be done using other solutions, such as NALC (N-Acetyl-L-Cysteine), -NaOH or other agents capable of digesting mucous material
For large volume liquid samples that have low or unknown bacterial loads e.g. urine, water collected from pool/river stream/tower	**Recommended pretreatment: Centrifugation** 1. Centrifuge the sample for up to 10 min at 20,000 × g to concentrate the bacterial cells as a pellet 2. Discard supernatant and resuspend the pellet in 220 µL Buffer BL2* 3. Aliquot 200 µL of suspension to the sample tube (supplied in the kit) *If sand or other particles are visible in the pellet, we recommend repeating centrifugation after BL2 buffer treatment or filtering out the dust
For cell-free body fluids e.g. CSF, BAL (Broncho-Alveolar Lavage), aspirates	**Recommended pretreatment: Centrifugation** **Method 1** 1. Pellet bacteria by centrifugation at 14,000 x g for 10 min 2. Resuspend bacterial pellet in 220 µL Buffer BL2 3. Transfer 200 µL of suspension to the sample tube (supplied in the kit) **Method 2 – Centrifugation-free** 1. Aliquot 200 µL sample in a 1.5 mL centrifuge tube 2. Add 200 µL buffer BL2 to sample (1:1) 3. Vortex, mixing for 5-10 seconds 4. Transfer 400 µL of sample to the sample tube (supplied in the kit)

Sample type	Procedure
For swab samples e.g. eye, nasal, pharyngeal, or other swabs	**Method 1** 1. Collect samples and place in 2 mL PBS containing a common fungicide. Incubate for 30 min at room temperature. 2. Pellet bacteria by centrifugation at 14,000 x g for 10 min 3. Resuspend bacterial pellet in 220 µL Buffer BL2 (supplied in the kit) 4. Transfer 200 µL of suspension to the sample tube (supplied in the kit) **Method 2 – Centrifugation-free** 1. Place the sample swab in 440 µL buffer BL2 and incubate for 30 min at room temperature 2. Transfer 400 µL to the sample tube
For select gram-positive bacteria species or samples that contain particles e.g. stool	**Recommended pretreatment: Mechanical homogenization** • Follow the standard homogenization procedures in the laboratory • For some sample types, DNA yield can be improved by performing this homogenization step prior to adding buffer BL2 and proteinase K
Isolation of genomic DNA from bacterial suspension cultures	1. Pipet 1 mL of bacterial culture into a 1.5 mL microcentrifuge tube and centrifuge at 5,000 x g for 5 min 2. Discard supernatant 3. Add 220 µL Buffer BL2 to pellet and mix by vortexing for 5-10 sec 4. Transfer 200 µL of suspension to the sample tube (supplied in the kit)
Isolation of genomic DNA from bacterial plate culture	1. Aliquot 1-3 bacterial colonies from culture plate with an inoculation loop and resuspend in 220 µL of buffer BL2 by vigorous stirring 2. Transfer 200 µL of suspension and transfer to the sample tube (supplied in the kit)
Inactive pathogenic organisms in the sample	**Recommended pretreatment: Boiling** 1. Incubate samples at 95°C for 10 min 2. Centrifuge briefly to collect the complete sample volume at the bottom of the tube 3. Allow samples to cool down or chill on ice, then transfer 100-400 µL cooled sample to the sample tube

Yield of Purified DNA

DNA yields depend on the sample type, number of bacteria in the sample and the protocol used for the purification of DNA.

Result

Scalability

MPure Bacterial DNA Extraction Kit was used to isolate the DNA from cultured *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC27154) in LB broth at different bacterial densities (measured Optical Density at 600 nm; OD_{600}). 200 µL of bacterial culture was aliquoted for extraction and the eluate was collected in 100 µL. Total nucleic acid yields of the bacterial densities were measured by Nanodrop 2000 UV-Vis spectrophotometer (Fig. 1a and 2a) and analyzed by 1% TAE agarose gel electrophoresis (Fig. 1b and 2b). The result shows that the nucleic acid extraction from both gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria have excellent scalability.

Fig. 1a. *E. coli*

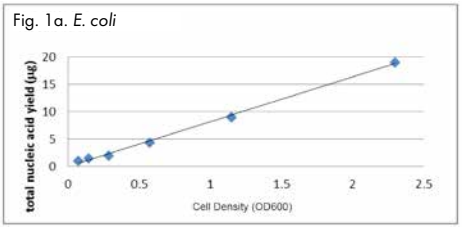


Fig. 1b

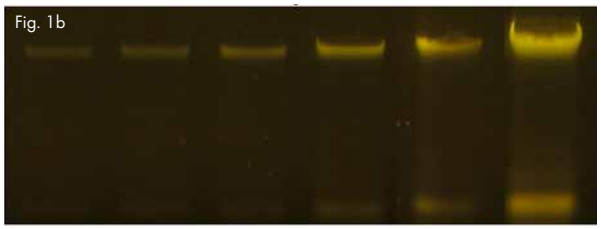


Fig. 2a. *Staphylococcus aureus*

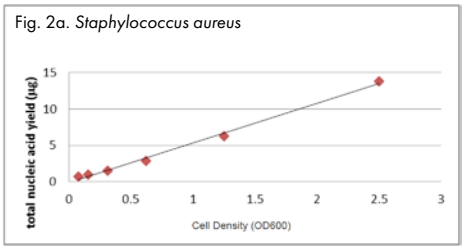
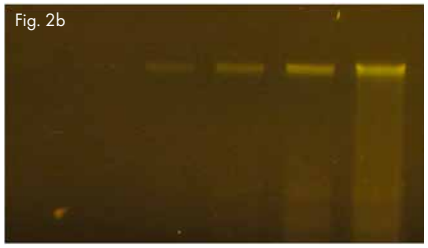


Fig. 2b



Sensitivity

Staphylococcus aureus (ATCC27154) samples were serially diluted in the range of 10^1-10^9 copy/mL. 200 µL samples were extracted and eluted in 100 µL. 25 µL of the eluate was used for SYBR Green real-time PCR reactions to detect the gene specific for *Staphylococcus aureus*. As little as 20 copies (about 10^2 copy/mL bacteria in the sample) of spiked-in (about 5 copies in the PCR reaction) bacteria can be detected, proving the excellent sensitivity and linearity of the isolation procedure (Fig. 3a and 3b)

Fig. 3a. Threshold

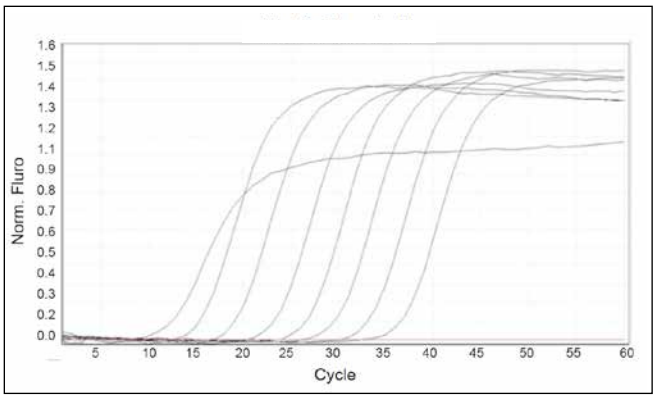
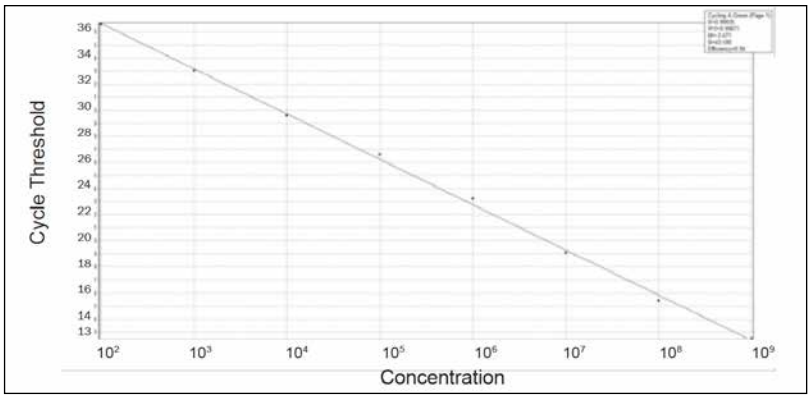


Fig. 3b.



Controls/Internal Control

Using appropriate controls for downstream analysis:

Type	Description	Location
Positive control	Use of a sample which is positive for the target	Place in sample tube
Negative control	Use of a sample which is negative for the target or water (NTC)	Place in sample tube
Internal control (IC)	Use of a defined quantity control	Place in sample tube or the round well of the reaction chamber

Limited Expressed Warranty Disclaimer

The manufacturer makes no expressed warranty other than that the test kit will function as a Research Use Only assay within the specifications and limitations described in the Instructions for Use and used in accordance with the instructions provided in the kit. The manufacturer disclaims any expressed or implied warranties with respect to merchantability, fitness for use or implied utility for any other purpose. The manufacturer's liability is limited.

Technical Problems

Should there be any technical problems, please do the following:

1. Note the kit lot number and the expiration date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.

MP Biomedicals

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