MPure Bacterial DNA Extraction Kit

Instructions For Use

CE

REVISION DATE: 2016-06
MSAH90031-ENG-1

REF 8AH90-048 (48 Tests)

TRADE NAME AND INTENDED USE

The **MP Diagnostics MPure Bacterial DNA Extraction Kit** is used with the **MPure-12 aNAP System** for extraction of genomic DNA from both Gram-positive and Gram-negative bacteria.

APPLICATION

Nucleic acids extracted from **MPure Bacterial DNA Extraction Kit** can be used in a number of downstream applications including: Polymerase Chain Reaction (PCR), quantitative PCR (qPCR), Next-Generation Sequencing (NGS), Microarray, Restriction Fragment Length Polymorphism (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 ISO 15223-1:2012.

	Use by	IVD	*In vitro* diagnostic medical device
	Temperature Limitation	LOT	Batch Code *Synonym:* Lot Number Batch Number
EC REP	Authorized representative in the European Community	Σ	Contains sufficient for <n> tests
	Consult Instructions for Use	REF	Catalogue Number Batch Code Synonym : Reference Number Re-order Number
	Manufacturer		

KIT COMPONENTS

Components	Quantity
CARTRIDGE RG **Reagent Cartridge**	48 pieces (24x2)
CHAMBER RX **Reaction Chamber**	48 pieces (24x2)
TIP HOL **Tip Holder**	48 pieces (24x2)
TIP FIL **Filter Tip**	50 pieces
PIN P **Piercing Pin**	50 pieces
TUBE SP **Sample Tube (2 ml)**	50 pieces
TUBE EL **Elution Tube (1.5 ml)**	50 pieces
BUF BL2 **BL2B Buffer**	1 bottle (25 mL)
Barcode Paper	1 copy
Selection Guide	1 copy
Instructions For Use	1 copy

REAGENT CARTRIDGE CONTENT

Well-1: Proteinase K solution = 40 µl
Well-2: Lysis Buffer 3 = 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 1 = 1000 µl
Well-9: Elution Buffer 2 = 1000 µl
Well-10: Empty

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For Professional use only.

Handling Requirements

1. Do not use kits beyond the expiry date.
2. Do not handle the reagents with bare hands. Avoid contact from your skin, eyes, or mucous membranes. If contact occurs, wash the affected area

immediately with large amounts of water. If spillage of the reagents occurs, dilute the spill with water before wiping it up.
3. Avoid mixing the reagents with sodium hypochlorite solution or strong acids. Otherwise, a highly toxic gas will be produced.

Laboratory Procedures
1. Treat all samples and waste as if potentially infectious, practice safe laboratory procedures. As sensitivity and titer of the pathogens in the sample varies, the operator needs to optimize the pathogen inactivation by boiling, using Lysis Buffer or taking the appropriate measures according to local safety regulations. MP Biomedicals does not warrant that samples treated with Lysis Buffer or boiling are completely inactivated or non-infectious. After sample processing, remove and autoclave all the disposable plastics.
2. Do not eat, drink or smoke in the laboratory working area.
3. Wear disposable gloves, laboratory coats and goggles when handling samples and kit reagents.
4. Do not use sharp or pointed objects when handling the reagent cartridges. This will prevent damage of the sealing foil and loss of reagent.
5. Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles.
6. Wash hands thoroughly after handling samples and test reagents.

Waste Handling
1. Discard unused reagents and waste according to country, federal, state and local regulations.

STORAGE

Store at room temperature (15-25°C). Do not freeze the reagent cartridges. The kits are stable for 18 months under the condition.

Store the purified total nucleic acid at 4°C (short-term, less than 10 days) or aliquot and store at -70°C (long-term) prior to performing the downstream analysis.

SPECIMEN COLLECTION, STORAGE AND TRANSPORT

Bacterial pellet/colony from culture, cell-free body fluids, liquid transport media, urine, environment material (water, soil, etc.)

When using the paraffin-embedded tissue sections as samples, it is recommended to extract DNA by **MPure FFPE DNA Extraction Kit** (**REF 8AJ30-048).**

When using tissue as samples, it is recommended to use the **MPure Tissue DNA Extraction Kit** (**REF 8AH70-048).**

The types and amounts of starting material for use in MPure Bacterial DNA purification procedures are shown as below:

Sample Type	Target Nucleic Acid	Sample volume (Amount of starting material)	Elution Volume
Bacteria Pellet	Genomic DNA	200-400µl /Up to 10^9 bacteria (about OD_{600} = 3)	50-300µl
Bacterial colony		200-400µl /1-3 colony	
Tissue		200-400µl /1-30 mg	
Urine		200-400µl /5-50 mL urine	
Cell-free body fluids		200-400µl cell-free body fluids	
Liquid transport media		200-400µl liquid transport media	
NOTE: Before extraction, adjust sample volume with BL2B buffer			

SPECIMEN PRETREATMENT

Sample preparation requirements are highly dependent on the type of starting material. Due to variations in consistency and viscosity, distinct handling is required.

The buffer BL2B is specialized for bacterial cell wall lysis* (supplied in the kit), it is used to resuspend the bacterial pellet before process 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** (**REF 8AJ20-048)**).

The table below describes the recommendations in processing the primary samples before nucleic acid extraction:

Sample type	Procedure
For viscous samples e.g. BAL, sputum or other mucous specimen	**Recommended pretreatment: Liquefaction** 1. Prepare a fresh DTT stock solution for liquefaction * (e.g., 5× conc. DTT stock is about 0.75%) 2. Adjust the final DTT concentration in the sample to 0.15% by adding DTT stock solution. 3. Incubate the sample (e.g. with shaking at 850r.p.m. for 30 min at 37°C) until it can be pipette easily.

	4. Pellet bacteria by centrifugation at 14000 x g for 10 min 5. Discard supernatant, resuspend the pellet in 220 µl Buffer BL2B 6. Transfer the 200µl suspension to sample tube (Supplied in the kit) * The liquefaction could be done by using other solutions, such as NALC（N-Acetyl-L-Cysteine）-NaOH or other agents which could digest 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 in pellet 2. Discard supernatant, resuspend the pellet in 220 µL Buffer BL2B* 3. Take 200 µL suspension to sample tube (Supplied in the kit) * If there were sand or other visible particle in the pellet, centrifuge again after BL2B buffer treatment or filter out the dust is recommended
For cell-free body fluids e.g. CSF, BAL, aspirates	**Recommended pretreatment: Centrifugation** Method 1 1. Pellet bacteria by centrifugation at 14000 x g for 10 min 2. Resuspend bacterial pellet in 220 µl Buffer BL2B 3. Take 200 µl suspension to sample tube (Supplied in the kit) Method 2-Centrifugation free 1. Take 200 µl sample in a 1.5 ml centrifuge tube 2. Add 200 µl buffer BL2B to sample (1:1) 3. Vortex for 5-10sec to mix 4. Transfer 400 µl sample to sample tube (Supplied in the kit)
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 30min at room temperature 2. Pellet bacteria by centrifugation at 14000 x g for 10 min 3. Resuspend bacterial pellet in 220 µl Buffer BL2B (Supplied in the kit) 4. Take 200 µl suspension to sample tube (Supplied in the kit) Method 2 - centrifuge free 1. Place the sample swab in 440 µl buffer BL2B, incubate for 30min at room temperature 2. Transfer 400 µl to sample tube
For some gram-positive bacteria species, especially for samples that contain particles e.g. stool	**Recommended pretreatment: Mechanical homogenization** • Follow the regular homogenization procedures in the laboratory. • For some sample types, DNA yield can be improved by performing this homogenization step prior to add buffer BL2B 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 5000 xg for 5 min 2. Discard supernatant 3. Add 220 µl Buffer BL2B to pellet and vortex for 5-10 sec 4. Take 200 µl suspension to sample tube (Supplied in the kit)
Isolation of genomic DNA from bacterial plate culture	1. Take 1-3 bacterial colony from culture plate with an inoculation loop and suspend in 220 µl of buffer BL2B by vigorous stirring 2. Take 200 µl suspension to sample tube (Supplied in the kit)
To 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 nucleated cells in the sample, and the protocol used for 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 density (measure the Optical Density at 600nm; OD_{600}). 200µl bacterial culture was used for extraction and 100µl eluate was collected. The total nucleic acid yield of different bacterial density was 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 shown that the nucleic acid extraction from both Gram-negative (*E.coli*) and Gram-positive (*S. aureus*) had excellent scalability.

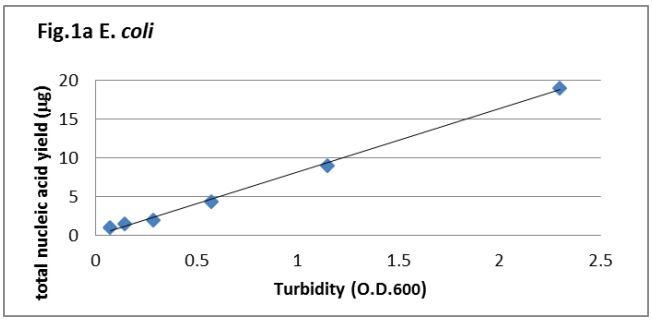
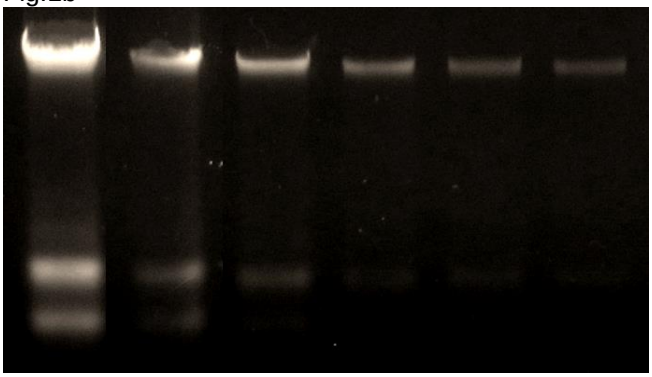


Fig.1b



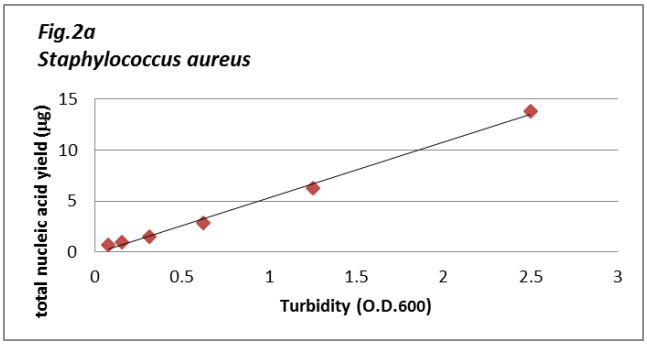
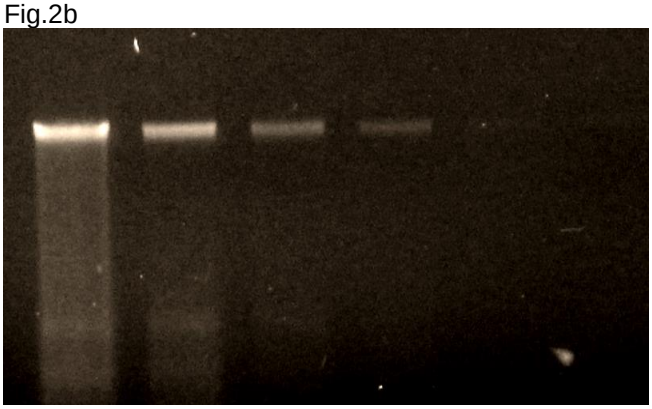


Fig.2b



Sensitivity

Serial-dilution on *Staphylococcus aureus* (ATCC27154) in range of 10^9-10^1copy/ml. 200µl sample were extracted and eluted in 100µl. 25µl eluate was used for SYBR Green real-time PCR reaction which detects *Staphylococcus aureus* specific gene. As little as 20 copies (about 10^2 copy/ml bacteria in the sample) spiked-in (about 5 copy in PCR reaction) bacteria could be detected. It proved that the excellent sensitivity and linearity of isolation procedure (fig. 3a and 3b).

Fig. 3a

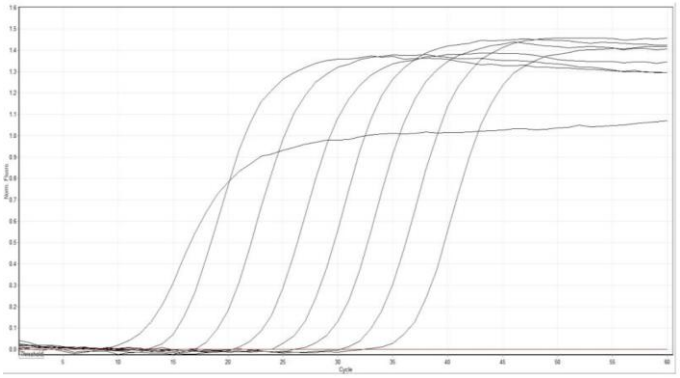
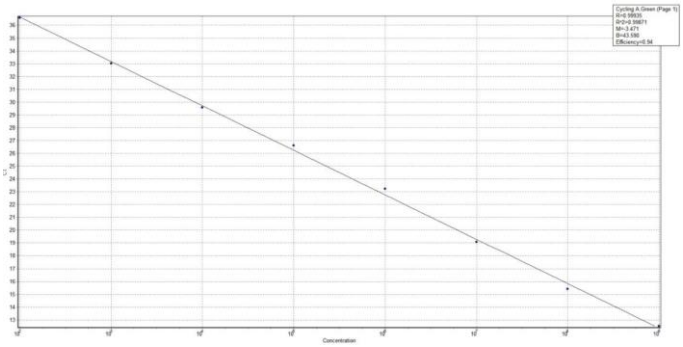


Fig. 3b



CONTROLS / INTERNAL CONTROL

Using appropriate controls for downstream analysis:

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

LIMITATION OF THE METHOD

The MPure Extraction Kits and the **MPure-12 aNAP System** are not intended for use as part of a specific in vitro diagnostic test. The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Appropriate controls must be included in any downstream diagnostic applications using nucleic acid purified using the **MPure-12 aNAP System** and the MPure Extraction Kits.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no expressed warranty other than that the test kit will function as an *in vitro* diagnostic assay within the specifications and limitations described in the Product Instructions For Use when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty, expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss howsoever caused by the product in the use or in the application thereof.

TECHNICAL PROBLEMS / COMPLAINTS

Should there be any technical problem / complaint, please do the following:

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

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