MPure Viral/Pathogen Nucleic Acids Extraction Kit B

Instructions For Use

CE

REVISION DATE: 2016-06
MSAJ60031-ENG-1

REF 8AJ60-048 (48 Tests)

TRADE NAME AND INTENDED USE

The **MP Diagnostics MPure Viral/Pathogen Nucleic Acids Extraction Kit B** is used with the **MPure-12 aNAP System** for extraction of viral and bacterial DNA/RNA from swab samples (cell-rich samples).

APPLICATION

Nucleic acids extracted from **MPure Viral/Pathogen Nucleic Acids Extraction Kit B** 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
RNA CA **RNA Carrier (1 mg)**	1 vial
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: BL2 Buffer = 400µl

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.

After dissolving the RNA carrier, store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the frozen RNA carrier more than 3 times.

Store the purified nucleic acid at 4°C (up to 24 hours) or at -20°C for longer storage. Repeated freeze–thawing is not allowed.

STARTING MATERIAL

- Bacterial pellet/colony from culture, clinical swab samples in liquid transport media, environment material (water, soil, etc.) and other cell-rich samples.

- If tissue or paraffin- embedded tissue sections (FFPE) are used as samples, it is recommended to extract the DNA by **MPure Tissue DNA Extraction Kit (REF 8AH70-048).**

- Types and amounts of starting material for use in **MPure Viral/Pathogen Nucleic Acids Extraction Kit B** purification procedures are shown as below:

Sample Type	**Target Nucleic Acid**	**Sample Volume (Amount of starting material)**	**Elution Volume**
Bacteria Pellet	Total Viral/Bacterial Nucleic acids (DNA/RNA)	100-200 µl /Up to 10^9 bacteria (about OD_{600} = 3)	50-300 µl
Bacterial colony		100-200 µl /1-3 colony	
Swab samples		100-200 µl liquid transport media	
Controls/ Internal Control*	Add controls /internal control in the extraction procedure if the downstream analysis needed (* see Controls/internal control)		

SPECIMEN PREPARATION

- Sample preparation requirements are highly dependent on the type of starting material. Due to variations in consistency and viscosity, distinct handling is required.
- Recommendations in processing the primary samples before nucleic acid extraction are shown as below:

Sample Type	**Procedure**
Inactivation of the pathogenic microorganism	Recommended pretreatment : Boiling 1. Incubate samples at 95°C for 10 min 2. Centrifuge briefly to collect the total sample volume at the bottom of the tube 3. Allow samples to cool down or chill on ice, then proceed with the following steps according to the sample type
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. shake at 850 r.p.m. for 30 min at 37°C) until it can be pipetted easily. 4. Transfer 200 µl to sample tube (provided in the kit)

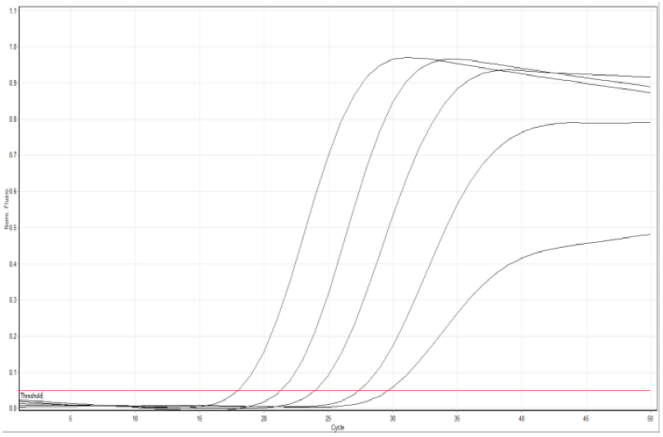
	* The liquefaction could be done by using other solutions, such as NALC（N-Acetyl-L-Cysteine） -NaOH or other agents which could digest mucous material
For large volume of liquid samples that have low or unknown bacterial loads e.g. urine, water collected from pool/river stream/tower	Recommended pretreatment : 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 PBS 3. Transfer 200 µl to a sample tube (provided in the kit)
Swab samples e.g. eye, nasal, pharyngeal, or other swabs	1. Collect samples and place in 1 ml PBS containing a common fungicide. Incubate for 30min at room temperature 2. Transfer 200 µl to a sample tube (provided in the kit)
For some gram-positive bacterial species. Especially for samples that contain particles e.g. stool	Recommended pretreatment : Mechanical homogenization Follow the regular homogenization procedures in the laboratory.
Bacterial suspension cultures	Transfer 200 µl culture to a sample tube (provided in the kit)
Bacterial colony	1. Take 1-3 bacterial colony from culture plate with an inoculation loop and suspend in 220µl PBS by vigorous stirring 2. Transfer 200 µl suspension to a sample tube (provided in the kit)

RESULTS

Nucleic acid yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for purification of nucleic acid.

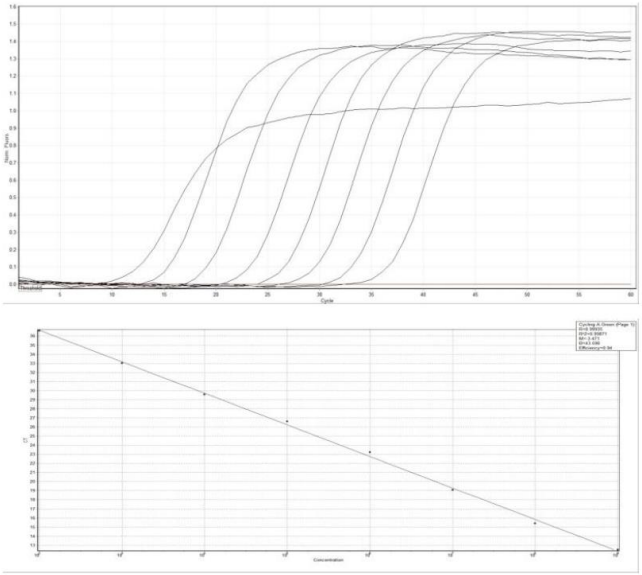
Sensitivity

Hepatitis B virus (HBV)



Using serum spiked with serial-diluted Hepatitis B Virus (in range of 300000-30 IU/ml). 200 µl serum sample were extracted and eluted in 100ul. 30 µl eluate was used for real-time PCR reaction.

Staphylococcus aureus



Performing serial-dilution on Staphylococcus aureus (ATCC27154) in range of 109-101copy/ml). As little of 20 copies (about 102 copy/ml bacteria in the sample) spiked-in (about 5 copy in PCR reaction) bacteria can be detected.

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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