USER MANUAL

For Research Use Only



MPure Viral/Pathogen Nucleic Acid Extraction Kit B

MPure Viral/Pathogen Nucleic Acids Extraction Kit B is used with the MPure 12 instrument for extraction of viral and bacterial DNA/RNA from viral, bacterial and swab samples (cell-rich samples).

Cat. No. **117022130** Size: **48 tests** Storage: **15–25 °C** Revision Date: **2022-08**



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INTRODUCTION

MPure Viral/Pathogen Nucleic Acid Extraction Kit B is used with the MPure-12 Automated Nucleic Acid Purification System to recover viral and bacterial RNA and DNA from swab samples (cell-rich samples). Nucleic acids extracted from MPure Viral/Pathogen Nucleic Acid 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.





Temperature Limitation



Batch Code (Synonyms: Lot Number, Batch Number)

KIT COMPONENTS

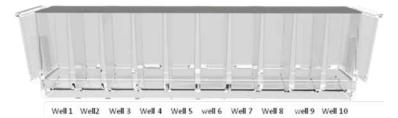
| Component Name | Quantity |
|-----------------------|--------------------|
| Reagent Cartridge | 48 pcs (4 x 6 x 2) |
| Reaction Chamber | 48 pcs (4 x 6 x 2) |
| Tip Holder | 48 pcs (24 x 2) |
| Filtered Tip | 50 pcs (25 x 2) |
| Piercing Pin | 50 pcs (25 x 2) |
| Sample Tube (2 mL) | 50 pcs (25 x 2) |
| Elution Tube (1.5 mL) | 50 pcs (25 x 2) |
| RNA Carrier (1 mg) | 1 pc |
| Instructions for Use | 1 pc |

STORAGE

Store at room temperature (15–25 °C). Do not freeze the reagent cartridges. The kits are stable for 12 months under these conditions.

After extraction, store the purified nucleic acids at 4 °C (short-term) or aliquot and store at -70 °C (long-term) before performing downstream analysis. Repeated freeze-thawing is not recommended.

REAGENT CARTRIDGE CONTENT



- Well 1: Proteinase K Solution (30 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 μ L) Well 7: Washing Buffer 3 (1000 μ L) Well 8: Elution Buffer 1 (1000 μ L) Well 9: Elution Buffer 2 (1000 μ L) Well 10: BL2 Buffer

STARTING MATERIAL

- This kit is designed for extraction of viral nucleic acids (e.g., those of HIV, HCV, HBV, CMV and EBV) from plasma or serum, or from a pool of cell-free body fluids.
- Bacterial pellet/colony from culture, clinical swab samples in liquid transport media, environment material (water, soil, etc.) and other cell-rich samples.
- If using tissue or paraffin- embedded tissue sections (FFPE) as samples, we recommend to extract DNA using the MPure Tissue DNA Extraction Kit (*Cat. No. 117022400*).
- The types and amounts of starting material for use in MPure Viral/ Pathogen Nucleic Acid Extraction Kit B purification procedures are shown in the table listed below:

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|-------------------------------|--|--|-------------------|
| Bacterial pellet | Total Viral Nucleic Acids (DNA + RNA) | 100-200 μL / Up to 10 ⁹ bacteria (about OD ₆₀₀ = 3) | |
| Bacterial colony | | 100-200 μL / 1-3 colony | 50-300 μL |
| Swab samples | | 100-200 μL liquid transport media | |
| Controls/ Internal control | ···· | | |

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

| Sample type | Procedure |
|---|---|
| Inactivation of the pathogenic microorganism | Incubate samples at 95 °C for 10 min Centrifuge briefly to collect the complete sample at the bottom of the tube Allow samples to cool down or chill on ice, then proceed with the following steps according to the sample type |
| Viscous samples e.g., Broncho- Alveolar Lavage, sputum or other mucous specimen | Recommended pretreatment: Liquefaction Prepare a fresh DTT stock solution for liquefaction* (e.g., 5× conc. DTT stock is about 0.75%) Adjust the final DTT concentration in the sample to 0.15% by adding DTT stock solution Incubate the sample (e.g., with shaking at 850 rpm for 30 min at 37 °C) until it can be pipetted easily Transfer 200 μL to sample tube (supplied) * The liquefaction can also be performed using other solutions, such as NALC (N-Acetyl-L-Cysteine) -NaOH or other agents which could digest mucous material |
| For large volume liquid samples with 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 a pellet 2. Discard supernatant, resuspend the pellet in 220 μL PBS 3. Aliquot 200 μL to a sample tube (<i>supplied</i>) |
| Swab samples e.g., eye, nasal, pharyngeal, or other swabs | Collect samples and place in 1 mL PBS containing a common fungicide; incubate for 30 min at room temperature Aliquot 200 μL to a sample tube |
| For select gram positive bacterial species, especially for samples that contain particles <i>e.g., stool</i> | Recommended pretreatment: Mechanical homogenization Follow the regular homogenization procedures in the laboratory. |
| Bacterial suspension cultures | Place 200 μL of the culture in the sample tube |
| Bacterial colony | Take 1-3 bacterial colonies from the culture plate with an inoculation loop and suspend in 220 μL PBS by vigorous stirring Place 200 μL suspension in the sample tube |

RNA CARRIER

Purposes of RNA Carrier during the purification procedure:

- Enhances binding of nucleic acids to the magnetic particles, especially for samples with low target molecules.
- The addition of RNA Carrier reduces the chances of RNA degradation in the rare event of residue RNases. If RNA carrier is not added to the reaction, recovery of DNA or RNA may be reduced.

RNA CARRIER INSTRUCTIONS FOR USE

- Add 1.0 mL RNase-free water to the lyophilized RNA Carrier (supplied) and mix by vortexing
- Store RNA Carrier at 4 °C (short-term, up to 1 month) or -20 °C (long-term). Do not freezethaw the frozen RNA carrier more than 3 times. Divide it into conveniently sized aliquots.
- Before nucleic acid extraction, it is recommended to add RNA Carrier to the sample. Add 5 μ L RNA carrier (for 100 μ L sample), 10 μ L (for 200 μ L sample) or 20 μ L (for 400 μ L sample) into the Sample Tube.

MPURE-12 PURIFICATION PROTOCOL

- 1 Turn on the MPure-12 instrument. Open the instrument door and remove the sample rack.
- 2 Load the Reagent Cartridge, then insert the Reaction Chamber, Tip Holder, Piercing Pins, and Filtered Tips into the instrument.
- 3 Insert the Sample tubes and Elution tubes into the Sample Rack.
- 4 Load the appropriate volume of sample into the sample tube, and place the sample rack back into the instrument.
- 5 Scan the protocol barcodes provided on the following page for the purification protocol, and the sample and elution volumes when prompted. Double check the selected options on the display screen to verify the appropriate program settings.
- 6 Press [ENTER] to start the program. At the end of the run, instrument will alarm briefly.
- 7 Open the instrument door and collect the elution tubes with the purified, ready-to-use nucleic acid. For short term, store at 4 °C. For long-term, store at -70 °C.
- 8 Discard all used cartridges and consumables into appropriate disposal.
- 9 Return the sample rack and close the door of the instrument.
- 10 Place in sleep mode by holding the "Start" button for 2 seconds or switch off the power to the instrument.

BARCODES FOR PURIFICATION PROTOCOL, SAMPLE VOLUME & ELUTE VOLUME SELECTION

NOTE Follow the instruction guide shown on MPure system's LCD screen to scan the barcodes.

1. Select Protocol

MPure Viral/Pathogen Nucleic Acid Extraction Kit B



2. Select Sample Volume

200 μL





Input sample volume by user via instrument control pad

3. Select Elute Volume

50 μ L200 μ LHHH<

Input elute volume by user via instrument control pad

QUALITY CONTROL

In accordance with MP Biomedicals' ISO-certified Quality Management System, each lot of MPure Viral/Pathogen Nucleic Acid Extraction Kit is tested against predetermined specifications to ensure consistent product quality.

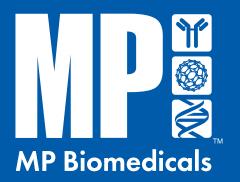
LIMITED EXPRESSED WARRANTY DISCLAIMER

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:

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



MP BIOMEDICALS

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