REF **ME-0044/ME-0045** **Model：MVR01**

Viral RNA Isolation Kit (Preloaded for Auto-Extraction)

Instructions for Use

ME-0044 Σ60

ME-0045 Σ240

15℃ 30℃

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I. Introduction

1、Intended Use

Viral RNA Isolation Kit (Preloaded for Auto-Extraction) utilizes magnetic particle technology for isolation and purification of pathogen's nucleic acids from biological specimens.

This kit can be used in combination with automated nucleic acid extraction systems. The product is intended to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

Viral RNA Isolation Kit (Preloaded for Auto-Extraction) is intended for *in vitro* diagnostic use.

2、Procedure Overview

With the effect of lysis buffer, the nucleic acids of pathogen were released and combined to Magnetic Cap. Then the Magnetic Cap with nucleic acids was transferred into washing buffer, proteins and other impurities were washed away. After that, Magnetic Cap was transferred into elution buffer,nucleic acids were eluted and transferred into centrifuge tube.

Note that this procedure recovers total nucleic acids, so if cells are present in the sample, cellular RNA/DNA will be recovered along with the viral RNA/DNA.

3、Kit Components

Component	Amount		Storage
	ME-0044 (60 preps)	ME-0045 (240 preps)	
Preloaded Plate（MVR01） ★a	5 pieces	20 pieces	RT
Magnetic Cap	5 strips	20 strips	RT
Proteinase K	1.3 mL×1	1.3 mL×4	RT
Carrier RNA ★b	1 tube	1 tube×4	RT
Carrier RNA Buffer	600 μL×1	600 μL×4	RT

Note： RT= Room Temperature (15–30°C)

★a Do not make Preloaded Plate frozen.

★b Lyophilized Carrier RNA can be stored at room temperature until the expiration date on the kit box. Add 500 μL of Carrier RNA Buffer to each vial of lyophilized Carrier RNA and mix well before use. Once Carrier RNA Buffer has been added into Carrier RNA, this Carrier RNA solution should be divided into conveniently sized aliquots which can be stored at 2°C～8°C for 6 months or -20°C for 2 years. The maximum number of freezing and thawing is 2.

4、Storage Conditions

Store at room temperature. The shelf life of the kit is 2 years. Both date of manufacture and the expiry date are indicated on the packaging.

5、Equipment and Reagents to Be Supplied by User

1	Automated Nucleic Acid Extraction Instrument (Liferiver, Cat. No. SB-Z-10011/Cat. No. SB-Z-10019)
2	1.5mL DNase/RNase-free tubes
3	Silicone Cover (Liferiver, Cat. No. OHC0143)
4	Normal Saline
5	Centrifuge
6	Vortex mixer

II. Isolation Protocol

1、Applicable Samples

1.1 Plasma: Collect 4mL whole blood into commercially available anticoagulant-treated tubes (Non-heparin anticoagulation). Cells are removed from plasma by centrifugation at 3000 rpm for 20 minutes at room temperature or by deposition at 4 ℃ for half an hour to one hour. The resulting supernatant is designated plasma. Transfer the liquid component (plasma) into a clean sterilization tube.

1.2 Serum: Collect 4mL whole blood into commercially available non-anticoagulant

tubes. After blood clot, cells are removed from serum by centrifugation at 3000 rpm for 20 minutes at room temperature or by deposition at 4 ℃ for half an hour to one hour. The resulting supernatant is designated serum. Transfer the liquid component (serum) into a clean sterilization tube.

Note: After blood collection, the separation must be accomplished in 6 hours. Recollect the blood sample if hemolysis was found. If white turbidity can be seen in plasma or serum samples, centrifugate at 13000 rpm for 5 minutes at 4 ℃ and transfer the plasma or serum using pipette.

1.3 Nasopharyngeal or oropharyngeal swab and nasal swab specimen:

1) If there is cell preservation solution in the sample collection tube, mix the tube by pulse-vortexing. The mixture solution is used for subsequent extraction.
2) If there is no preservation solution in the sample collection tube, add 1 mL of normal saline to the tube, mix the tube by pulse-vortexing. The mixture solution is used for subsequent extraction.

Samples can be transported using ice box or foam box with dry ice. Samples can be stored at 2℃～8℃ for less than 3 days. With the long-term preservation, store them under -70℃ and avoid repeating freezing and thawing.

1.4 Sputum specimen: Use a sterile sputum collector to collect 1-5 mL of sputum from the deep lungs of the examinee, and seal it for examination. Add an equal volume of sputum treatment solution, screw the tube cap tightly, mix well for a few seconds, incubate at room temperature for 30 minutes to fully liquefy the sample. Gently flick the collector to concentrate the sputum sample at the bottom of the tube for subsequent nucleic acid extraction.

2、Reagents peparation

2.1 Proteinase K：Always mix well before use and store it at room temperature.

2.2 Carrier RNA: Before first use of Carrier RNA, add 500 µL Carrier RNA Buffer to each vial of lyophilized Carrier RNA and mix well. Divide this Carrier RNA solution into conveniently sized aliquots, and store them at 2℃～8℃ for 6 months or at -20°C for 2 years. The maximum number of freezing and thawing is 2.

2.3 Preloaded Plate: Centrifuge briefly before use to confirm the solution of E was in the bottom of the plate.

3、Isolation Procedure

3.1 Procedure for full plate (12 samples)

3.1.1 Take out one piece of preloaded plate and tear the aluminum foil cover carefully.

3.1.2 Add 300 µL of sample, 20 µL of Proteinase K and 6 µL of Carrier RNA into each well A1–A12 of the preloaded plate.
Note: Internal Control (IC) can be added in this step but the use of it should follow the instruction of detection kit for use of Internal Control. If necessary, Carrier RNA, Proteinase K and IC can be mixed before adding.

3.1.3 Put the preloaded plate on the transport platform carefully, and insert the magnetic cap.

3.1.4 According to the user manual of Automated Nucleic Acid Extraction Instrument, choose “RNA Isolation 2” program, then press “START” to run.

3.1.5 After the program has been finished, discard the magnetic cap, take out the preloaded plate and transfer the liquid in well E1–E12 into 1.5 mL DNase/RNase-free tubes. It can be used immediately or stored at -20°C or -80°C for preservation.

3.2 Procedure for less than 12 samples

3.2.1 Take out one piece of preloaded plate and tear the aluminum foil carefully.

3.2.2 Add 6 µL of Carrier RNA、20 µL of Proteinase K and 300 µL of sample into the corresponding well A of the preloaded plate. For example, if there are totally 5 samples, add 6 µL of Carrier RNA、20 µL of Proteinase K and 300 µL of sample into the well A1–A5 of the preloaded plate separately.

3.2.3 Put the preloaded plate on the transport platform carefully, and insert the magnetic cap.

3.2.4 According to the user manual of Automated Nucleic Acid Extraction Instrument, choose “RNA Isolation 2” program, then press “START” to run the test.

3.2.5 After the program has been finished, discard the magnetic cap, take out the preloaded plate and transfer the liquid in the corresponding well E into 1.5 mL DNase/RNase-free tubes. It can be used immediately or stored at -20°C or -80°C for preservation. For example, samples are added into well A1–A5, then collect the solution in well E1–E5.

3.2.6 Cover the preloaded plate timely with the Silicone Cover (not provided, Cat. No. OHC0143). For the second use, open the silicone cover and operate according to step 3.2.2–3.2.5.

Note: Do not use the same preloaded plate for more than 2 times, and the interval between 2 runs must shorter than 24 hours.

III. Appendix

1、Performance Index

It is verified that the Viral RNA Isolation Kit (Preloaded for Auto-Extraction) is applicable for the extraction of pathogens from samples, which include Hepatitis C

virus (HCV) and Rubella virus (RV).

2、Warnings & Precautions

1) Clean and disinfect the workbench before starting the experiment.
2) Wear disposable gloves (change constantly) and use disposable centrifuge tubes, pipettes and filter tips.
3) Biological cabinet (negative pressure) or anti-contamination cover should be used during experimental operation in order to prevent environmental contamination.
4) This experiment needs to be carried out by skilled operator.
5) Regularly clean and disinfect workbench and pipettes etc. With 10% hypochlorous or 75% ethanol, and use UV lamp or ozone to disinfect them for half an hour to one hour.
6) The reagents should be mixed at room temperature before use.
7) Don’t mix up reagents of different lots. The kit shall be used within shelf life period.
8) Improper transportation and storage of clinical samples may lead to poor extraction efficiency or even failure.
9) If reagents spill on skin, rinse with water immediately please.
10) All containers, reagent bottles, package and the remaining samples should be disposed of as medical waste.