REF **ME-0077** **Model: LVR01**

Viral RNA Isolation Kit (Centrifuge Column)

Instructions for use

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Ⅰ. Introduction

1. Intended Use

The Viral RNA Isolation Kit (Centrifuge Column) utilizes silica-membrane technology for isolation and purification of Viral RNA from fluid specimens, such as plasma, serum, lymph, etc.

The product is intended to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

The Viral RNA Isolation Kit (Centrifuge Column) is intended for *in vitro* diagnostic use.

2. Procedure Overview

After the samples containing the target nucleic acid are lysed by the lysis buffer, high purity nucleic acid is obtained by washing, eluting and purifying process based on the principle of specific and efficient combination of silica gel membrane and nucleic acid.

3. Kit Components

Ref.	Components	Amount	Storage
1	Binding buffer (LVR01)	30mL	RT
2	Washing buffer A (LVR01)	13mL	RT
3	Washing buffer W (LVR01)	12mL	RT
4	Elution buffer	15mL	RT
5	Carrier RNA	310μg	RT
6	Carrier buffer	1mL	RT
7	Binding Columns (LVR01)	50	RT
8	Collection Tubes（2mL）	50	RT
9	Centrifuge Tubes（1.5mL）	50	RT

Note: RT= Room Temperature (15–30°C)

★Don't mix up reagents of different lots.

★Add 310μL Carrier buffer to the tube of lyophilized Carrier RNA and mix well before first use. After dissolution, the solution should be stored at -20°C.

★Add 17 mL absolute ethanol to the bottle of Washing buffer A and mix well.

★Add 48 mL absolute ethanol to the bottle of Washing buffer W and mix well.

4、Storage Condition

Store at room temperature. The shelf life of the kit is 12 months. Both manufacture and the expiry date are indicated on the packaging.

5、Equipment and Reagents to Be Supplied by User

1	Absolute ethanol
2	Centrifuge
3	1.5mL DNase/RNase-free tubes
4	Vortex mixer
5	Pipettes

Ⅱ. Isolation Protocol

1、Applicable Samples

1.1 Applicable sample type: Plasma, serum, lymph, etc.

1.2 Sample collection: Samples should be collected according to the regular sample collection method.

1.3 Sample storage and transportation: The test samples can be processed immediately after collection, or it can be long-term stored under the condition of -20°C/-80°C. Repeated freezing and thawing should be avoided. The samples should be transported in foam box filled with ice.

2、Reagent Preparation

2.1 Please ensure that Washing buffer A and Washing buffer W have been prepared with appropriate volume of absolute ethanol as indicated on the bottle.

2.2 **Carrier RNA solution:** Add 310μL Carrier buffer to the tube containing 310 μg lyophilized Carrier RNA to obtain a solution of 1 μg/μL. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times.

Note: Lyophilized Carrier RNA should not be dissolved in Binding buffer directly. It must firstly be dissolved in Carrier buffer and then added to Binding buffer.

2.3 **Carrier RNA working solution:**

2.3.1 Calculate the volume of Binding buffer/Carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from table 1. Mix Binding buffer/Carrier RNA mix upside down to obtain the Carrier RNA working solution. To avoid foaming, do not use vortex oscillation.

2.3.2 For larger numbers of samples, volumes can be calculated using the following sample calculation:

n×0.56mL = y mL

y mL× 10 μL/mL = z μL

n = number of samples to be processed simultaneously

y = calculated volume of Binding buffer

z = volume of Carrier RNA/ Carrier buffer to add to Binding buffer

Table 1 Volumes of Binding buffer and Carrier RNA solution required for the Carrier RNA working solution

No. of samples	Vo.Binding buffer（mL）	Vo.Carrier RNA/Carrier buffer（μL）	No. of samples	Vo.Binding buffer（mL）	Vo.Carrier RNA/Carrier buffer（μL）
1	0.56	5.6	13	7.28	72.8

2	1.12	11.2	14	7.84	78.4
3	1.68	16.8	15	8.40	84.0
4	2.24	22.4	16	8.96	89.6
5	2.80	28.0	17	9.52	95.2
6	3.36	33.6	18	10.08	100.8
7	3.92	39.2	19	10.64	106.4
8	4.48	44.8	20	11.20	112.0
9	5.04	50.4	21	11.76	117.6
10	5.60	56.0	22	12.32	123.2
11	6.16	61.6	23	12.88	128.8
12	6.72	67.2	24	13.44	134.4

3、Isolation Procedure

1) Pipette 560μL of Carrier RNA working solution (mixture of Binding buffer and Carrier RNA solution, the preparation method is shown in Table 1 or calculated according to the formula) into a clean 1.5mL centrifuge tube (self prepared).

Note: If the sample volume is larger than 140μL, increase the amount of Binding buffer-Carrier RNA proportionally.

2) Add 140μL plasma, serum or lymph (equilibrate the samples to room temperature (15-30°C)) to the Binding buffer-Carrier RNA in the centrifuge tube. Mix by pulse-vortex for 15 s.

Note: To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Carrier RNA working solution to yield a homogeneous solution.

3) Incubate at room temperature (15-30°C) for 10 min.
4) Briefly centrifuge the tube to remove drops from the inside of the lid.
5) Add 560μL of absolute ethanol to the sample, and mix by pulse-vortex for 15 s.

Note: Cool absolute ethanol on ice before use if the room temperature is more than 30°C.

6) Briefly centrifuge the tube to remove drops from inside the lid.
7) Carefully transfer the 630μL lysate onto the Binding Column in a 2mL Collection Tube without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000×g) for 1 min. Discard the filtrate; place the Binding Column in the same collection tube.

Note: If the lysate has not completely passed through the Binding Column after centrifugation, centrifuge again at higher speed until the Binding Column is empty.

8) Repeat the previous step with the remaining lysate in the centrifuge tube.
9) Carefully open the Binding Column, and add 500μL of Washing buffer A (Ensure that absolute ethanol has been added before use) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000×g) for 1 min. Discard the filtrate and place the Binding Column in the same collection tube.
10) Carefully open the Binding Column, and add 500μL Washing buffer W (Ensure that absolute ethanol has been added before use) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000×g) for 1 min. Discard the filtrate and place the Binding Column in the same collection tube.
11) Repeat the previous step.
12) Place the Binding Column in the same collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 3 min to dry the membrane completely.

Note: Ethanol carryover into the eluate may cause problems in downstream applications.

13) Optional: Place the Binding Column into the same 2mL collection tube (not provided), open the lid, and incubate at room temperature (15-30°C) for 3 min to dry the membrane completely.
14) Place the Binding Column in a clean 1.5 mL Centrifuge Tube, and discard the collection tube with the filtrate. Carefully open the lid of the Binding Column, and apply 60μL of Elution buffer to the center of the membrane. Close the lid and incubate at room temperature (15-30°C) for 5 min. Centrifuge at 8,000 rpm (~6,000×g) for 1 min.

Note: Ensure that the elution buffer is equilibrated to room temperature (15-30°C) before use. If elution is done in small volumes (<50μL), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA. Adjust the volume of elution buffer according to the requirements of specific experiments. Incubate at room temperature (15-30°C) for 5 min to increase the RNA yield after Elution buffer is added into the Binding Column.

15) The quality of the extracted DNA is detected by real-time PCR.

Ⅲ.Appendix

1、Performance Index

It is verified that the Viral RNA Isolation Kit (Centrifuge Column) is applicable for the nucleic acid extraction of viral RNA from samples, such as Plasma, serum, lymph, etc. It is equivalent to QIAamp Viral RNA Mini Kit in isolation efficiency.

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 to avoid 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 0.5–1 hour.
6) Don't mix up reagents of different lots. The kit shall be used within shelf life period.
7) Improper transportation and storage of clinical samples may lead to poor extraction efficiency or even failure.
8) All containers, reagent bottles, package and the remaining samples should be disposed of as medical waste.
9) If precipitate has formed in Binding buffer or Washing buffer A, dissolve the buffer in 37°C water bath and mix thoroughly before use.
10) All centrifugation steps should be carried out at room temperature.
11) Centrifuge Tubes (1.5mL) are used in 3.14. Others are not supplied.
12) If the solution contacts with skin and mucous membrane, please wash it with tap water immediately, which will not cause harm to the operator.
13) After using the solution, tighten the cap to avoid evaporation.