For research use only Version Number: 2.0-2001

Viral RNA Isolation Kit

For viral RNA from buccal swab, plasma, serum, cell-free body fluids, cell-culture supernatants

Component	RE-01011	RE-01014
	50T	200T
Linear Acrylamide	120 μl	480 μl
Buffer viRL*	25 ml	100 ml
Buffer viRW1	25 ml	100 ml
Buffer viRW2	24 ml	96 ml
RNase-Free ddH_2O	10 ml	40 ml
RNA-Only Column	50	200
Manual	1	1

*: Buffer viRL contains irritating chaotropic salt, please wear gloves and take relevant protective measures during operation.

Introduction

This kit uses the spin column and formula developed by our company, which can efficiently isolate and purify viral RNA from samples such as buccal swabs, plasma, serum, cell-free body fluids and cell culture supernatants. The kit is specially added with Linear Acrylamide, which can easily capture trace RNA from the system, and has the characteristics of convenience, high yield, and good repeatability.

The whole system is RNase-Free, so that the extracted viral RNA is not degraded; Buffer viRW1, Buffer viRW2 buffer washing system makes the obtained RNA extremely pure.

Storage and Stability

- This kit (except linear acrylamide) can be stored for 24 months under dry conditions at room temperature (15-25°C); if it needs to be stored for longer, it can be stored at 2-8°C.
- Linear Acrylamide solution is recommended to be stored frozen at 2-8℃，and can be stored at -20°C; after adding Linear Acrylamide to Buffer viRL, it can be stored at 2-8°C for up to 48h. Please use it now.

Note: If stored at low temperature, the solution is prone to precipitation. Before use, be sure to place the solution at room temperature for a period of time. If necessary, preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate, and mix it before use.

Yield and Quality

The viral RNA extraction kit can be used to purify viral RNA from samples such as buccal swabs, plasma, serum, cell-free body fluids, and cell culture supernatants. Its output is related to the sample itself, the initial volume of the sample, the freshness of the sample, and the preservation of the sample. Time and operation related. The purified RNA has an OD260/280=1.8-2.1.

Warnings and Precautions

- All experimental steps should be performed at room temperature (15-25°C) (including centrifugation). **Do not use ice bath and low-temperature (4°C) centrifugation.**
- The sample should avoid repeated freezing and thawing, otherwise the extracted miRNA will be degraded and the extraction amount will also decrease.
- The processing volume of liquid samples should not exceed 200 μl, otherwise it will affect the yield and purity of viral RNA.
- After adding Linear Acrylamide to Buffer viRL, it can be stored at 2-8°C for up to 48h. **Please use it as soon as possible**.
- Before use, add absolute ethanol to Buffer viRW2 according to the label on the bottle.
- RNA yield and quality are related to the elution volume and sample processing volume. It is recommended to use sample volume per 500μl Buffer viRL: 200μl liquid sample, 1 buccal swab.
- elution volume: The volume of the eluate should not be less than 30 μl, otherwise it will affect the efficiency of RNA recovery.
- Please check the Buffer viRL in the kit for crystals. If crystals are precipitated after low-temperature storage, you can put the Buffer at room temperature or 37°C for a period of time, dissolve the crystals and mix well before using.

Procedure (Operate at room temperature (15-25°C))

Before use， please add absolute ethanol to Buffer viRW2 according to the label on the bottle.

1. Prepare fresh lysis buffer：add 500 μl Buffer viRL and 2 μl Linear Acrylamide to a clean 2ml tube, and mix by inversion.

   Note: To avoid foaming in the solution, do not vortex. As the number/volume of samples increases, the added amount of Buffer viRL and Linear Acrylamide solutions will be enlarged in equal proportions.

2. Sample processing

2a. Liquid sample: Add 200 µl plasma/serum/cell-free body fluid/cell culture supernatant to the centrifuge tube in step 1 (the sample needs to be equilibrated to room temperature). Vortex for 15 sec. In order to ensure sufficient lysis, the sample and the lysis buffer need to be thoroughly mixed.

2b. Oral swab: Cut off the cotton swab from a sample taken from the oral swab, place it in the tube of step 1, and vortex.

3. Incubate for 10 min at room temperature (15-25°C).
   Notice: If the sample is a buccal swab, discard the buccal swab after incubation.

4. Centrifuge briefly to collect the liquid adhering to the tube wall and tube cap.

5. Add 350 µl isopropanol, and vortex for 15 sec.

6. Centrifuge briefly to collect the liquid adhering to the tube wall and tube cap.

7. Carefully transfer 750µl of the mixture above to the RNA-Only Column (with the collection tube), centrifuge at 8,000 rpm (~6,000 × g) for 1 min, and discard the flow-through.
   Note: If flocculent precipitate appears in the mixture, please transfer the precipitate to the RNA-Only Column. If all the liquid on the adsorption column cannot be centrifuged into the collection tube, please increase the speed and extend the centrifugation time until the liquid is completely transferred to the collection tube.

8. Put the RNA-Only Column back into the same collection tube, repeat step 7 until all the remaining mixture has been processed.

9. Add 500 µl Buffer viRW1 to the RNA-Only Column, centrifuge at 8,000 rpm (~6,000 ×g) for 1 min, and discard the flow-through.

10. Add 700 µl Buffer viRW2 to the RNA-Only Column **(make sure to add absolute ethanol according to the instructions before use)**, centrifuge at 8,000 rpm (~6,000 × g) for 1 min, and discard the flow-through.

11. Repeat step 10 one more time。

12. Put the RNA-Only Column back into the collection tube, centrifuge the empty tube at 12,000 rpm (~13,400 ×g) for 2 minutes, and discard the collection tube.

13. Transfer the RNA-Only Column to a new tube, and add 30-50µl RNase-Free ddH2O preheated at 65°C to the center of the RNA-Only Column membrane **(do not add the eluate to the pressing ring)**, and incubate at room temperature for 2 minutes. Centrifuge at 12,000 rpm (~13,400 ×g) for 1 min to collect the RNA solution.
    Note: The volume of RNase-Free ddH2O added should not be less than 30 µl. If the volume is too small, it will affect the elution efficiency. To increase the RNA yield, add the RNA solution obtained by centrifugation to the RNA-Only Column again, and repeat step 13. The purified RNA can be used directly in downstream experiments or stored at -80°C.