RNA Isolation Kit（Paramagnetic Beads Column）

Instruction Manual

LT0100EM

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Ⅰ. Introduction

1、Product Description

The Viral RNA Isolation Kit is designed for rapid and automatic purification of viral RNA from samples such as serum, plasma, gargle, hydrothorax, ascites, C.S.F. and urine samples.

The microspherical paramagnetic beads used in the kit have a large available binding surface and can be fully dispersed in solution, allowing thorough nucleic acid binding, washing, and elution. The procedure, therefore, delivers very consistent yields of high quality RNA with little sample-to-sample variation.

2、Overview of the Procedure

Paramagnetic beads with a nucleic acid binding surface are then added to the sample to bind nucleic acids. The beads/nucleic acids are captured on magnets, and proteins and other contaminants are washed away. The beads are then washed again to remove residual binding solution. Nucleic acids are eluted in a small volume of elution solution. Note that this procedure recovers total nucleic acids, so if cells are present in the sample, cellular RNA will be recovered along with the viral RNA. The RNA recovered with the kit is of high quality and purity, and is suitable for real-time PCR or real-time RT-PCR.

3、Kit Components and Storage Conditions

The RNA Isolation Kit contains reagents to isolate Viral RNA or Cellular RNA 50 samples.

Amount	Component		Storage
50	Binding Columns		room temp
26ml	Binding Buffer		room temp
52ml	Washing Buffer A		room temp
14ml	Washing Buffer W		room temp
	(Add 42ml 100% ethanol before use)		
6ml	Elution Buffer		room temp
1.1ml	RNA Binding Beads A	★a	room temp
350μl	Carrier RNA	★b	room temp
	(Add 350μl 100% Elution Buffer before use)		
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★a Don't make **RNA Binding Beads A** frozen

★b Once Elution Buffer has been added to Carrier RNA, store at 2℃~8℃ for up to 6 months and at-20℃for up to 24 months.

Store at room temperature for 24 months from the date received.

4、Required Materials Not Provided With the Kit

Reagents 100% ethanol, AR grade or better

1.5ml RNase-free tubes

Ⅱ. Isolation Protocol

Viral RNA Isolation Protocol

1、 Equipment and Reagent Preparation

- **RNase precautions**

Lab bench and pipettors

Before working with RNA, it is always a good idea to clean the lab bench and pipettors.

Gloves and RNase-free technique

Wear laboratory gloves for this procedure; they protect you from the reagents, and they protect

the RNA from nucleases that are present on skin.
Use RNase-free pipette tips and tubes to handle the kit reagents, and avoid putting used tips into the reagent containers.

- **Add Carrier RNA、RNA Binding Beads A to the Binding Buffer**

Add Carrier RNA、RNA Binding Beads A to Binding Buffer Concentrate according to the table below, and mix briefly. This mixture is called **Binding Solution** in these instructions.

Binding Solution	**Per Test**
Binding Buffer	500ul
Carrier RNA	6ul
RNA Binding Beads A	20ul

We recommend including ~10% overage to cover pipetting error when preparing the **Binding Solution.** Vortex the RNA Binding Beads A at moderate speed to form a uniform suspension before pipetting.

- **Add ethanol to the Washing Buffer W, mix**

Add 42 ml 100% ethanol to the bottle labeled as Washing Buffer W before use and mix well. Mark the label to indicate that the ethanol was added.

2、Viral RNA Isolation Procedure

Sample type

It is designed for isolation of viral RNA from cell-free, or nearly cell-free samples. For example, biological fluids such as serum, plasma, milk, urine, meconium, and nasal fluids can be used. Other common sample types such as spent culture medium and swab samples are also compatible.

Sample volume

With up to 140μl sample input, the Viral RNA Isolation procedure can be completed in the 1.5ml tubes.

- **Add 140μl sample to 526μl Binding Solution**

a. Pipet 526μl prepared Binding Solution into each 1.5ml **RNase-free** tube.
b. Transfer 140μl sample to the Binding Solution in the **RNase-free** tube.
When adding sample, immerse pipette tips slightly in the Binding Solution to prevent creating aerosols that can lead to cross-contamination.
c. Mix by gently vortexing for 10 s or by flicking the tube 5~10 times.
d. settle for 3 min at room temperature

- **Capture the RNA with Binding Beads A**

a. Transefer the 666μl solution above mentioned to a Binding Column
b.Centrifuge the tube at 13000rpm for 60sec
c. Discard the solution in the collection tube below.

- **Wash Twice with 500μl washing Buffer A**

a. Add 500μl Washing Buffer A to each sample
b. Centrifuge the tube at 13000rpm for 40sec.
c. Discard the liquid in tne collection tube below.
d. Repeat steps a–c to wash with twice 500μl of Washing Buffer A.

- **Wash Twice with 500μl Washing Buffer W**

a. Add 500μl Washing Buffer W to each sample
b. Centrifuge the tube at 13000rpm for 15sec.
c. Discard the liquid in the collection tube.
d. Repeat steps a–c to wash with twice 500μl of Washing Buffer W.

- **Dry the beads by centrifuging**
  Centrifuge the binding columns at 13000rpm for 2min.
  It is important to avoid overdry the RNA Binding Beads A because this may make the beads more difficult to resuspend in the next step.
- **Elute the RNA in 50μl Elution Buffer**
  a. Put the binding column on a 1.5ml RNase-free tube. Add 50μl Elution Buffer (**prewarmed to 65°C**) to each sample, mix through tips to make beads completely dissolved in Elution Buffer, and settle for 2 min at room temperature.
  b. Centrifuge the tube at 13000rpm for 2min, and store the purified RNA in the 1.5ml RNase-free tube at –20°C.

Operation Diagram

1. Add 140μl sample to 526μl Binding Solution
2. Gently invert for 10 sec , and settle for 3 min

3. Transefer the 666μl solution above to a Binding Column
4.Centrifuge the tube at 13000rpm for 60sec
5. Discard the solution in the collection tube.

6.Wash twice with 500μl Washing Buffer A and Wash twice with 500μl Washing Buffer W
7. Dry the beads by centrifuging the binding columns at 13000rpm for 2min.

8. Elute the RNA in 50μl Elution Buffer

9. Pure Viral Nucleic acid

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