Ver: 2211

Virus RNA Miniprep Kit

（BW-R6620）

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Options: Removal of genomic DNA using DNase digestion ..... 错误！未定义书签。

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Kit Contents

Catalog#	BW-R6620-00	BW-R6620-01	BW-R6620-02
Preps	4	50	250
Buffer LY	2.4 mL	28 mL	135 mL
Buffer RB	3 mL	40 mL	185 mL
RNA Wash Buffer	2 mL	24 mL	3 x 24 mL
DEPC-Treated ddH2O	500 µL	10 mL	30 mL
RNA Columns	4	50	250
2 mLCollection Tubes	8	100	500
1.5 mL RNase-free tube	4	50	250
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* Add 8 mL (BW-R6620-00) or 96 mL (BW-R6620-01) or 96 mL (BW-R6620-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

The Virus RNA Miniprep Kit provides an easy and reliable method for isolating total viral RNA from plasma or serum while enzyme inhibitors and other contaminates completely removed. This procedure has been tested for isolating nucleic acids from Hepatitis A, Hepatitis B, Hepatitis C and HIV. This kit can also be used to isolate viral DNA/RNA from urine and cell culture supernatant.

Storage and Stability

All components can be stored at 4-28℃. All kit components are guaranteed for 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

Important Notes

✪Crystals may form in Buffer LY, dissolve the precipitates at 37 oC before use.

✪Add 8 mL(BW-R6617-00) or 96 mL (BW-R6617-01) or 96 mL (BW-R6617-02) 100% ethanol to each RNA Wash Buffer before use.

✪Determine the volume of Buffer LY to be used and add 20 µL of β - mercaptoethanol (β -ME) per 1 mL Buffer LY before use. Buffer LY contains β -ME can be stored at room temperature for up to 1 month

Materials not Supplied

✪Tabletop microcentrifuge .

✪100% ethanol

✪Vacuum manifold if use vacuum protocol.

Note: Perform all steps including centrifugation at room temperature

Protocol For Total Viral RNA Extraction From Plasma Or Serum

1. Pipet 100-300 μL plasma or serum into a 1.5 mL tube and add **2 volumes Buffer LY** into the plasma or serum.

   Note: Add 20 μL of β-mercaptoethanol per 1 mL Buffer LY before use.
2. Mix it thoroughly by vortexing and incubate for 10 min at room temperature.
3. Add 0.5 volume 100% ethanol into the lysate (for example: 250 μL 100% ethanol for 500 μL lysate) and pipet 5 times to mix the solution.
4. Transfer the solution into a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.
5. Add **500 μL Buffer RB** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.
6. Add **650 μL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.

   Note: Ensure that ethanol has been added to RNA Wash Buffer before use.
7. Add another **650 μL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, with the lip open, back to a new collection tube.
8. Centrifuge the column at 13,000 rpm for 2 min. Discard the flow-through.

   Note: It is critical to remove residual ethanol for optimal elution.
9. Place the column to a RNase-free 1.5 mL tube, add **30-50 μL DEPC-treated ddH_2O**(Can be reduced to 15 ~30 μL) to the column and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20℃.

Protocol For Total Viral RNA Extraction From Stool

1. Suspend 0.5–1.0 mL stool in up to 5 mL 0.89% NaCl (i.e., up to 1:10 dilution).
2. Clarify the solution by centrifugation for 20 min at 4000 x g.
3. Filter the supernatant using a 0.22 μm filter.
4. Use **140 μL the filtrate** as starting material (Page 2)following the Protocol For Total Viral RNA Extraction Protocol.

   Note: Stool, plasma, serum, urine, cerebrospinal fluid, bone marrow, and other body fluids often contain only very low numbers of cells or viruses. In these cases we

recommend concentrating the samples (as large as 3.5 ml) to a final volume of 200 μl, by ultrafiltration.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An A260/A280 ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

Trouble Shooting Guide

Problem	Possible reason	Suggested Improvement
Low A260/A280 ratios	Protein contamination	Do a Phenol: Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
	Guanidine Thiocyanate contamination	Add 2.5 volumes of ethanol and 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20℃. Centrifuge at 10,000 g for 15 min at 4℃.Resuspend the RNA pellet in DEPC-treated water.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -70℃. after collect it.
	The binding capacity of the membrane in the spin column was exceeded	Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield.
	Ethanol not added to buffer	Add ethanol to the RNA Wash Buffer before purification.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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