Ver: 2002

EZgene™ Blood Viral DNA/RNA Miniprep Kit

（BW-VR6511）

浙杭械备 20200101

Contents

Kit Contents

Catalog#	BW-VR6511-00	BW-VR6511-01	BW-VR6511-02
Preps	4	50	250
Buffer LY	5 mL	30 mL	130 mL
L Solution	50 µL	120 µL	520 µL
Wash Buffer *	1 mL	12 mL	50 mL
Buffer RB	5 mL	30 mL	130 mL
DEPC-treated ddH_2O	500 µL	10 mL	30 mL
MV Micro Columns	4	50	250
2 mL Collection Tubes	8	100	500
1.5 mL RNase-free Microfuge Tubes	4	50	250
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*Add 4 mL (BW-VR6511-00) or 48 mL (BW-VR6511-01) or 200 mL (BW-VR6511-02) 96-100% ethanol to each Wash Buffer bottle before use.

Introduction

The EZgene™ Viral DNA/RNA kit provides an easy and reliable method for isolating total viral DNA/RNA from plasma, serum, whole blood, urine and cell culture supernatant. This procedure has been tested for isolating nucleic acids from Hepatitis A, Hepatitis C and HIV. The isolated DNA/RNA can be used for PCR, RT-PCR and other downstream applications.

Storage and Stability

L Solution store at -20℃. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 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

✪Add 4 mL (BW-VR6511-00) or 48 mL (BW-VR6511-01) or 200 mL (BW-VR6511-02) 96-100% ethanol to each Wash Buffer bottle before use.

✪Calculate and aliquot amount of Buffer LY to be used in a clean tube and add 1% volume of β-mercaptoethanol to Buffer LY or 20μL DTT (2M) to 1 mL Buffer LY. Add 4 μL of L Solution per 1 mL of Buffer LY/β-me. Mix well.

Materials not Supplied

✪Tabletop microcentrifuge.

✪96-100% ethanol.

✪1.5 mL sterile tubes.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

Protocol for Viral DNA/RNA Extraction

The protocol is developed for 150 µL samples. Small samples should be adjusted to 150 µl with phosphate-buffered saline (PBS) before loading, and samples with a low viral titer should be concentrated to 150 µl before processing. For samples 150 -300 µL, the amount of Buffer LY buffer and other reagents should be increased proportionally, but the amounts of Buffers RB and Wash Buffer used in the wash steps do not need to be increased.

1. Prepare a master mix of **Buffer LY/β-me/L Solution** as described. The mixture is stable at 2-8°C for 48 hours.

2. Pipet **150 µL** plasma, serum, cell free body fluid or other samples into a 1.5 mL tube and add **500 µL Buffer LY/β-me/L Solution** into sample.
   Note: Ensure that β-mercaptoethanol or DTT has been added to Buffer LY before use.

3. Mix it thoroughly by vortexing and incubate for 10 minutes at room temperature.

4. Add 1 volume **100% ethanol** into the lysate (for example: 500 µL 100% ethanol for 500 µL lysate) and pipet 5 times to mix the solution.

5. Transfer the solution into the **MV Micro Column** and centrifuge at 12,000 rpm for 1 minute. Discard the **2 mL Collection Tube** with the flow-through and put the column back to a new collection tube.

6. Add **500 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.

7. Add **500 µL Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.

8. Optional: Repeat step **7**.

9. Centrifuge the empty column, with the lid open, at 12,000 rpm for 2 minute.

   Note: It is critical to remove residual ethanol for optimal elution

10. Place the column to a **1.5 mL RNase-free Microfuge Tube**, add **35-50 µL DEPC-treated**

ddH_2O to the column and centrifuge at 12,000 rpm for 1 minute. Store the purified DNA/RNA at -20°C.

11. Optional: Add the eluent back to the column for a second elution.

Note: The first elution normally yield 60-70% of the DNA/RNA while the second elution yield another 20-30% of the DNA/RNA bound to the column.

Protocol: Sample Concentration

Plasma, serum, urine, cerebrospinal fluid, bone marrow, and other body fluids often have very low viral titers. In these cases, concentrating samples of up to 5 ml to a final volume of 150 µL is recommended.

Important point before starting

Use centrifugal filter such as Amicon-4, UFC810024, Microsep 100, Filtron: 3.5 ml, cat. no. OD100C40, Ultrafree®-CL, or equivalent from other suppliers.

Procedure

1. Apply up to 4 ml of sample to the filter unit following the manufacturer's instructions.

2. Centrifuge according to manufacturer's instructions to a final volume of 150 µl.

Some samples, plasma in particular, may be difficult to concentrate to 150 µl due to high viscosity. Centrifugation for up to 2 hours may be necessary.

3. Pipet 150 µl of concentrated sample into a 1.5 ml microcentrifuge tube, and follow the Viral RNA Mini Spin Protocol.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low A_{260}/A_{280} ratios	Protein contamination	Do a Phenol:Chloroform extraction. Loss of total RNA(up to 40%) should be expected.
Low A_{260}/A_{280} ratios	Guanidine Thiocyanate contamination.	Add 2.5 volumes of ethanol and 0.1 M NaCl (final concentration) to precipitate RNA. Incubate for 30 minutes at -20℃. Centrifuge at 13,000 rpm for 15 minutes at 4°C. Resuspend the RNA pellet in DEPC-Treated ddH_2O.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -80℃ after collect it.
Low Yield	The binding capacity of the membrane in the spin column was exceeded	Use of too much sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield.
Low Yield	Ethanol not added to buffer	Add ethanol to the Wash Buffer.
Genomic DNA contamination	Too much total RNA sample was used in RT-PCR.	Reduce total RNA amount used in RT-PCR to 50-100 ng.

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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