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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

All other components can be stored at room temperature(15-25℃）. All kit components are guaranteed for 1 year from the date of production.

Kit Contents

Catalog#	**VR6512-00**	**VR6512-01**	**VR6512-02**
Preps	1	10	25
Buffer PLY	22 mL	210 mL	500 mL
Wash Buffer *	0.5 mL	3 mL	7mL
DEPC-Treated ddH_2O	110 µL	1.1 mL	2.6 mL
Mini Columns	1	10	25
Collection Tubes	1	10	25
Instruction Booklet	1	1	1

*Add 2 mL (VR6512-00) or 12 mL (VR6512-01) or 28 mL (VR6512-02) 100% ethanol into each Wash Buffer bottle before use.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- Add 1% volume of β-mercaptoethanol to Buffer PLY before use and store at 4 °C.
- Add 2 mL (VR6512-00) or12 mL (VR6512-01) or 28 mL (VR6512-02) 100% ethanol into each Wash Buffer bottle before use.

Materials supplied by users

- Tabletop microcentrifuge
- 1.5 mL sterile tubes
- 100% ethanol

Note: Perform all steps including centrifugation at room temperature

Protocol for Viral DNA/RNA extraction

1. Pipet **8-10 mL** plasma, serum or other samples into a 50 mL tube and add **2 volumes** of **Buffer PLY**.

   Note: Ensure that β-mercaptoethanol has been added to Buffer PLY.
2. Mix it thoroughly by vortexing and incubate for 15 min at room temperature.
3. Add **0.5 volume 100% ethanol** into the **lysate** (for example: **10 mL** 1**00% ethanol** for **20 mL lysate)** and pipet 5 times to mix the solution.
4. Insert a Mini column into a collection tube. Transfer the solution into Mini column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.
5. Add **600 µL Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.
6. Repeat step **5**.
7. Discard the flow-through liquid ,reinsert the column to the collection tube. Centrifuge the empty column, **with the lid open,** at 13,000 rpm for 2 min.

   Note: This step removes residual ethanol for optimal elution.
8. Place the column to a RNase-free 1.5 mL tube, add **50-100 µL DEPC-Treated ddH_2O** to the column and centrifuge at 13,000 rpm for 1 min to elute the viral DNA/RNA (Store viral RNA at -20^{o}C and viral DNA at 4 ^{o}C).

Trouble Shooting Guide

Problem	Possible reason	Suggested Improvement
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 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20°C. Centrifuge at 13,000 rpm for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -70°C 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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