APEX SCIENTIFIC
Contact: Andy Pepper 086 2577641
andy@apexscientific.ie

Viral RNA Extraction from Respiratory Specimens

Contents

Introduction

The EZgene™ Viral DNA/RNA mini kit provides an easy and reliable method for isolating total viral RNA from plasma, serum, nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates, and sputum. This procedure has been tested for isolating nucleic acids from Hepatitis A, Hepatitis C and HIV. The isolated RNA can be used for PCR, qRT-PCR and other downstream applications.

Storage and Stability

All other components can be stored at room temperature. All kit components are guaranteed for 1 year from the date of purchasing.

Kit Contents

Catalog#	VR6568-00	VR6568-01	VR6568-02
Preps	4	50	250
Buffer LY	5 mL	30 mL	130 mL
L Solution	50 µL	120 µL	520 µL
Proteinase K (20 mg/mL)	90 µL	1.1 mL	5.5 mL
RNA 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
Mini Columns	4	50	250

Caution: Buffer LY and Buffer RB contain chaotropic salts, wear gloves and protection eyewear when handling these buffers.

Prepare RNA Wash Buffer

VR6568-00: Add 4 mL 100% ethanol to RNA Wash Buffer before use.

VR6568-01: Add 48 mL 100% ethanol to RNA Wash Buffer before use.

VR6568-02: Add 200 mL 100% ethanol to RNA Wash Buffer before use.

Before Start

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

Important

- ☑ Calculate and aliquot amount of Buffer LY to be used in a clean tube and add 1% volume of β-mercaptoethanol to Buffer LY. Add 4 µL of L Solution per 1 mL of Buffer LY/β-me. Mix well.
- ☑ Prepare RNA Wash Buffer as described.

Note: Perform all steps including centrifugation at room temperature

Materials supplied by users

- ☑ Tabletop microcentrifuge and 1.5 mL RNase free tubes.
- ☑ 100% ethanol.

Viral RNA Isolation

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 PHB and RNA 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 on page 3. The mixture of Buffer LY/RNA carrier is stable at 2-8 ℃ for 48 hours.

2. Pipet **150 µL** plasma, serum, cell free body fluid or other samples into a 1.5 mL tube and add **0.5 mL Buffer LY/β-me/ L Solution**.

3. Add **20 µL Proteinase K**, mix thoroughly by vortexting and incubate at 30°C for 20 min. Proteinase K is necessary for extracting RNAs from nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates, and sputum.

4. Add **1 volume 100% ethanol** into the lysate and pipet 5 times to mix the solution.

5. Transfer the solution into a RNA column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

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

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

8. Centrifuge the empty column at 12,000 rpm for 2 min.
   It is critical to remove residual ethanol for optimal elution.

9. Place the column to a RNase-free 1.5 mL tube, add 35-50 **µL DEPC-treated water** to the column and centrifuge at 12,000 rpm for 1 min. The viral DNA/RNA is in the flow-through liquid. Store the purified DNA/RNA at -20℃.

10. 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.

Trouble shoot 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 10,000 g 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 liability

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga 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 at (858) 603-3219 or visit our website at www.biomiga.com