Biomiga Inc.
The Inventor of EZgene™ and ViraTrap™ Systems

Viral DNA/RNA Extraction from Respiratory Specimens

VER 210518.V5

Contents

Safety Information

Strictly follow CDC or Depart of Health guidance for handling infectious samples. Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens. Specimen processing should be performed in a certified biological safety cabinet accordingly following biosafety guidelines for the specific virus.

Buffer LYE and Buffer RB contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste, wear gloves and protective eyewear when handling.

When transferring a column/collection tube, always hold the collection tube, not the column, to prevent the collection tube falling off.

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Introduction

The EZgene™ Viral DNA/RNA mini kit provides an easy and reliable method for isolating total viral DNA/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 COVID-19, Hepatitis A, Hepatitis C and HIV. The isolated RNA can be used for PCR, qRT-PCR and other downstream applications.

Storage and Stability

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

Kit Contents

Catalog#	VR6568-01	VR6568-02
Preps	50	250
Buffer LYE	25 mL	90 mL
Proteinase K (25 mg/mL)	1 mL	5 mL
RNA Wash Buffer *	12 mL	50 mL
Buffer RB	20 mL	90 mL
L Solution (RNA Carrier)	210 µL	1,100 µL
DEPC-Treated ddH_2O	5 mL	15 mL
Mini Column with collection tubes	50	250
Collection tubes	4x50	4x250
Vacuum connecters (Optional)	50	250

Before Start

Important points before starting

 Always use RNase-free equipment.

 Always change pipet tips between liquid transfers. To minimize cross contamination, we recommend the use of aerosol-barrier pipet tips.

 All centrifugation steps are carried out at room temperature (15–25°C).

 Always use disposable gloves and regularly check that they are not contaminated with sample material. Discard gloves if they become contaminated.

 This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.

Important

- ☑ Add 200 mL (VR6568-02) or 48 mL (VR6568-1) 100% ethanol to RNA Wash Buffer before use.
- ☑ Add 40 mL (VR6568-02) or 9 mL (VR6568-01) isopropanol to Buffer RB before use.

Materials supplied by users

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

Handling RNA

Since RNases are difficult to inactivate, only use RNase free consumables for experiment. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

- Aseptic technique should always be followed when extracting with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination.
- Change gloves frequently and keep tubes closed whenever possible.
- Work through protocol carefully and quickly to avoid RNA degradation.

Viral RNA Isolation Protocol

The protocol is developed for processing 50-300 µL samples.

1. Pipet **10 µL Proteinase K, 4 µL L Solution,** and **300 µL Buffer LYE** to a 1.5 mL tube.
   Calculate the number of samples to be processed and make master mix of proteinase K, L Solution and Buffer LYE.

2. Pipet **300 µL** nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates, or plasma, serum, into the 1.5 mL tube from Step 1. Mix well by vortexting for 10 seconds. Incubate at room temperature for 2-5 minutes to lyse the cells and viruses.

3. Add **600 µL isopropanol** and mix well by vortexing for 5 seconds.
   Note: Maintain the ratio of (Sample+ Buffer LYE): Isopropanol= 1:1

4. Transfer **600 µL** of the sample from step 3 into a RNA column and centrifuge at 10,000 rpm for 30 seconds. Discard the collection tube contains the flow-through and transfer the column to a new collection tube. Apply the remaining sample to the column and spin at 10,000 rpm for 30 seconds.

5. Discard the collection tube with the flow-through and transfer the column to a new collection tube. Add **500 µL Buffer RB** to the column and centrifuge at 10,000 rm for 30 seconds. Discard the collection tube contains the flow-through and transfer the column to a new collection tube.

6. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 10,000 rpm for 30 seconds. Discard the collection with the flow-through and transfer the column to a new collection tube.

7. Centrifuge the empty column at 14,000 rpm (maximum speed) for 2 min. **It is critical to remove residual ethanol for optimal elution.**

8. Transfer the RNA column to a RNase-free 1.5 mL tube, add 35-50 **µL DEPC-treated water** directly to the center of the column membrane and centrifuge at 10,000 rpm for 30 seconds. The viral RNA is in the flow-through liquid.

9. Optional: Add the eluent back to the column membrane for a second elution.
   Note: The first elution normally yields 70% of the RNA while the second elution yields another 20-30% of the RNA bound to the column.
   Note: The purified RNA should be put on ice for downstream application or store at -80°C or -20°C.

Vacuum manifold protocol

1. Prepare samples by following the protocol on page 3, step 1-3. Prepare vacuum manifold according to manufacturer's instructions. Insert a disposable connecter to the manifold and a RNA column to the connecter.

2. Pipet **600 µL** sample/LYE/Proteinase K/L Solution/Isopropanol mixture to the column. Switch on vacuum source to draw the sample through the column. Process the remaining sample mix as described.

3. Wash the column by adding **500 µL Buffer RB**; draw the Buffer RB through the column by the vacuum force.

4. Wash the column by adding **500 µL RNA Wash Buffer;** draw the wash buffer through the column by the vacuum force.

5. Turn off the vacuum according to manufacturer's instruction. Transfer the column to the collection tube and proceed to step 7 on page 3.

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 collection.
Low Yield	The binding capacity of the membrane in the spin column was exceeded	Use of too much of the sample and exceeding the binding capacity of the 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.

For technical support or learn more product information, please contact us at (858) 603-3219 or visit our website at www.biomiga.com