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

Pathogen DNA/RNA Extraction

VER 220810

Contents

Strictly follow CDC /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 contain chaotropic salts, wear gloves and protection eyewear when handling these buffers. DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Due to the shortage of supplies, use the columns and collection tubes from the same kit only. The columns and collection tubes may not be compatible across different kits.

When transferring the column/collection tubes, always hold the upper rim of the collection tube, not the column, to avoid possible drop off of the collection tube.

CE IVD

For *in vitro* diagnostic

Introduction

The EZgene™ Pathogen DNA/RNA extraction kit provides an easy and reliable method for isolating pathogen DNA/RNA from plasma, serum, nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates, sputum and lesion swab. This procedure has been tested for isolating nucleic acids from COVID-19, Monkeypox, Hepatitis A, Hepatitis C and HIV. The isolated DNA/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#	VD6518-01	VD6568-02
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
DEPC Water	5 mL	15 mL
Mini Column with collection tubes	50	250
Collection tubes	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.

¬ To minimize cross-contamination, open only one tube at a time.

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

Important

- ☑ Add 200 mL 100% ethanol to RNA Wash Buffer before use.
- ☑ Add 40 mL isopropanol to Buffer RB before use.

Materials supplied by users

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

Handling DNA/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.

Pathogen DNA/RNA Isolation Protocol

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

1. Pipet **20 µL Proteinase K,** 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 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 pulse-vortexting for 10 seconds. Incubate at room temperature for 5-10 min to lyse the cells and virus.

3. Add **600 µL isopropanol** and mix well by pulse-vortexing for 5 seconds. Spin briefly to collect the drop from the lid.
   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 flow-through carefully to a waste container by pipetting and put the column back to the collection tube. Transfer the remaining sample to the column and centrifuge at 10,000 rpm for 30 seconds. Discard the collection tube and transfer the RNA column to a new collection tube. **Note: When transferring the column/collection tubes, always hold the upper rim of the collection tube, not the column, to avoid possible drop off of the collection tube.**

5. Add **500 µL Buffer RB** to the column and centrifuge at 10,000 rm for 30 seconds. Discard the flow-through. Put the column back to the collection tube.

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

7. Centrifuge the empty column at 12,000 rpm 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 Water** to the column and centrifuge at 10,000 rpm for 30 seconds. The viral DNA/RNA is in the flow-through liquid.

9. Optional: Add the eluent back to the column for a second elution.
   Note: The first elution normally yields 70% of the DNA/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 -20°C.

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.

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

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