

# EpiMag™ Viral RNA Isolation Kit (Magnetic Beads)

Base Catalog # P-9108

## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

**Uses:** The EpiMag™ Viral RNA Isolation Kit is suitable for a quick preparation of viral RNA with magnetic beads from cell-free liquid specimens (excluding plasma and serum), specifically from saliva and nasal or nasopharyngeal swabs.

**Input Amount:** The amount of starting materials can be up to 400 µl of liquid volume, with the best volume of 200 µl. A total of 50 standard extractions (using 200 µl of sample) can be performed with this kit.

**Binding Capacity and Yield:** The beads binding capacity can be up to 5 µg. RNA spiking tests show that the yield is >90%. However, the yield from different samples may vary depending on the sample type.

**Purity:** Purified RNA is ready for most downstream applications and specifically for RT-PCR.

**Precautions:** To avoid cross-contamination, carefully pipette the sample or solution using aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

## KIT CONTENTS

Components	50 samples P-9108-050	Shipping Temperature	Storage Upon Receipt	Storage Checklist
<b>SRS</b> (Sample Release Solution)	16 ml	RT	RT	
<b>RLB</b> (RNA Lysis Buffer)	10 ml	RT	RT	
<b>RPB</b> (RNA Purification Buffer)	7 ml	RT	RT	
<b>RWB</b> (RNA Wash Buffer)	8 ml	RT	RT	
<b>EB</b> (Elution Buffer)	2 ml	RT	RT	
<b>Magbeads</b>	250 µl	RT	4°C	

## SHIPPING & STORAGE

The kit is shipped at ambient room temperature. Upon receipt: Store the **Magbeads** at 4°C away from light and all other components at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

**Note:** Check all buffers for salt precipitation prior to use. Re-dissolve any precipitate by warming up to 37°C.

## MATERIALS REQUIRED BUT NOT SUPPLIED

- Vortex mixer
- Magnetic device (1.5 ml microtube format)
- Pipettes and RNase-free pipet tips
- 1.5 ml microcentrifuge tubes and 0.2 ml PCR tubes (RNase-free)
- Isopropanol (100%)
- Ethanol (100%)
- Distilled water

## GENERAL PRODUCT INFORMATION

**Quality Control:** Each lot of the EpiMag™ Viral RNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality. EpiGentek guarantees the performance of all products in the manner described in our product instructions.

**Product Warranty:** If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

**Safety:** Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

**Product Updates:** EpiGentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

**Usage Limitation:** The EpiMag™ Viral RNA Isolation Kit is for research use only and is not intended for diagnostic or therapeutic application.

## A BRIEF OVERVIEW

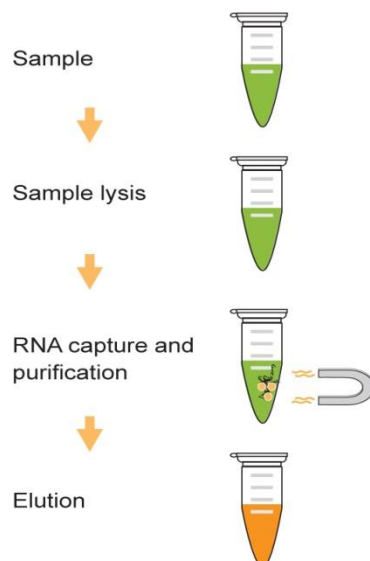
The *EpiMag™ Viral RNA Isolation Kit* provides a fast, simple, and cost-effective method for the isolation of viral RNA from cell-free liquid specimens (excluding plasma and serum), specifically from saliva and nasal or nasopharyngeal swabs. The specialized buffering system allows RNA to bind to the magnetic beads while contaminants and impurities are efficiently washed away, and pure RNA is eluted. The RNA purified with the EpiMag™ Viral RNA Isolation Kit is suitable for a variety of routine applications, specifically for RT-PCR.

The kit has the following features:

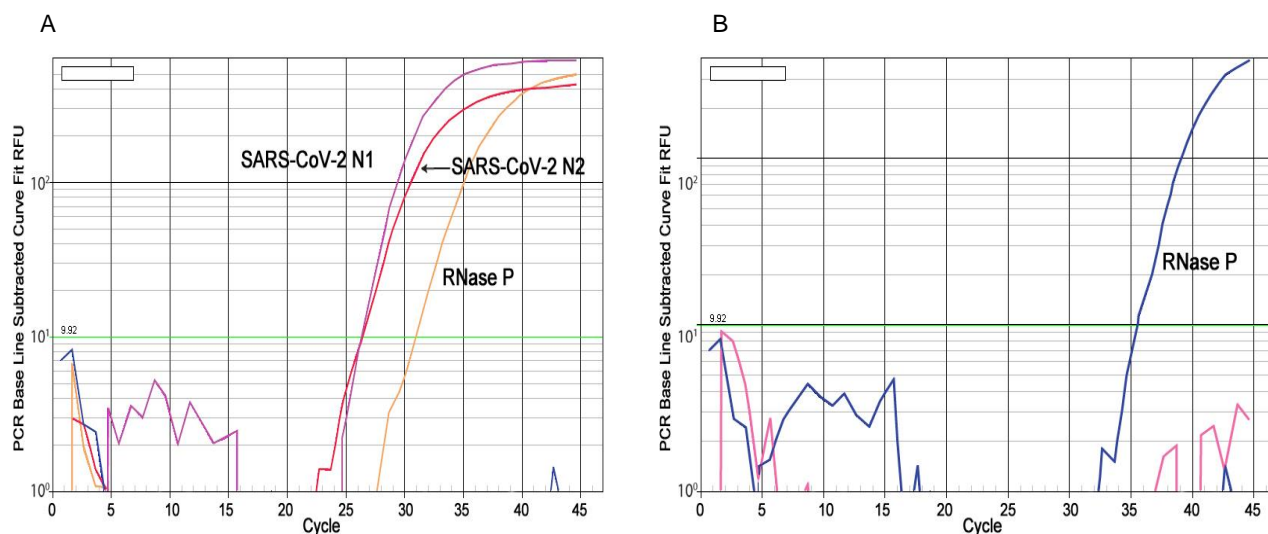
- Fast procedure delivers high-quality total RNA in total 25 minutes
- Allowing small size (>30 bases) and large size (<200 kb) to be isolated
- Convenient handling without the need for centrifugation
- Ready-to-use RNA for high performance in downstream application
- Consistent RNA yield from a small amount of starting material

## PRINCIPLE & PROCEDURE

The EpiMag™ Viral RNA Isolation Kit contains all the reagents required for successfully performing RNA isolation directly from cell-free samples (excluding plasma and serum). After lysis, binding, and wash, RNA is easily recovered in quantities of up to 5 µg using specially designed magnetic beads. Total RNA is then ready to be used for a variety of downstream applications.



**Fig 1.** Schematic Procedure for the EpiMag™ Viral RNA Isolation Kit.



**Fig 2.** Total RNA was isolated from nasal swab samples with EpiMag™ Viral RNA Isolation Kit and was used for RT-PCR analysis. A: Nasopharyngeal swab sample was spiked with SARS-CoV-2-N positive control; B: Nasopharyngeal swab sample was not spiked with SARS-CoV-2-N positive control. PCR was processed with use of primers and probes against SARS-CoV-2 N1/N2 and human RNase P gene (internal control).

## PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

### Starting Materials

*Input Amount:* The sample amount can be up to 400 µl volume.

### Working Buffer Preparation

**Working RWB (RNA Wash Buffer):** Add 32 ml of 100% ethanol to 8 ml of **RWB (RNA Wash Buffer)**

### 1. Sample Harvesting

Liquid viral sample:

- 1.1a Directly collect 200 µl of liquid samples into a 1.5 ml vial.
- 1.2a Add 100 µl of the **RLB (RNA Lysis Buffer)**. Mix by pipetting.
- 1.3a Incubate at room temperature for 5 min.

Swab sample:

- 1.1b Correctly collect nasal or nasopharyngeal swab samples according to the established swab sample collection instruction.
- 1.2b Place the swab into a clean 1.5 ml microtube, and snap off the handle. Add 300 µl of **SRS** (Sample Release Solution) and rotate the swab in **SRS** for 30 seconds.

- 1.3b Remove the swab and discard. Add 100  $\mu$ l of the **RLB (RNA Lysis Buffer)** to the sample.
- 1.4b If the transport of the swab sample is needed, the swab sample should be eluted in 200-300  $\mu$ l of the transport media. Add 200  $\mu$ l of the transport media containing swab sample into a 1.5 ml microtube and then add 100  $\mu$ l of the **RLB (RNA Lysis Buffer)** to the sample. Mix by pipetting.
- 1.5b Incubate at room temperature for 5 min and meanwhile re-suspend magbeads by shaking or vortexing.

## **2. RNA Isolation**

- 2.1 After incubation, add 70  $\mu$ l of **RPB (RNA Purification Buffer)** followed by adding 300  $\mu$ l of isopropanol and 4  $\mu$ l of re-suspended magbeads. Mix the sample by inverting gently the tube or by pipetting up and down at least 10 times, and then incubate at room temperature for 5 min.
- 2.2 Put the tubes in the magnetic device until the solution is clear (about 4 min). Carefully remove and discard the supernatant.
- 2.3 Keep the tubes in the magnetic device and add 250  $\mu$ l of working **RWB (RNA Wash Buffer)** to the tubes, then carefully remove and discard the supernatant.
- 2.4 Repeat step 2.3 once for a total of two washes.

**Note:** If more than 200  $\mu$ l of sample are used, then step 1 and 2 should be repeated in increments of 200  $\mu$ l of the sample and add buffers/reagents proportionally (i.e., if 400  $\mu$ l of sample is being used, 200  $\mu$ l of **RLB (RNA Lysis Buffer)** should be added at step 1.2a, 1.3b, or 1.4b. 140  $\mu$ l of **RPB (RNA Purification Buffer)**, 600  $\mu$ l of isopropanol and still 4  $\mu$ l of magbeads should be added at Step 2.1. Then go to the Step 2.2.

## **3. Elution**

- 3.1 Keep the tubes in the magnetic device to dry the beads for 1 min.
- 3.2 Resuspend the beads in 20  $\mu$ l of **EB (Elution Buffer)**, and incubate at room temperature for 5 min to release the purified RNA from the beads.
- 3.3 Capture the beads by placing the tubes in the magnetic device until the solution is completely clear.
- 3.4 Transfer 20  $\mu$ l from each sample to a new 0.2 ml PCR tube for immediate use or store at  $-20^{\circ}\text{C}$  for up to 2 months or at  $-80^{\circ}\text{C}$  for long term.

**Note:** For high concentrated RNA use 10  $\mu$ l to elute

## TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Degraded RNA/low integrity	RNase contaminant	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free tubes.
Low yields of RNA	Poor binding	Ensure sufficient volume of RPB (RNA Purification Buffer) and isopropanol is added. And ensure <b>working RWB (RNA Wash Buffer)</b> is properly prepared.
	Incorrect elution conditions	Add 10-20 $\mu$ l of the <b>EB (Elution Buffer)</b> to the beads, incubate for 5 minutes, and then pull down the beads.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Repeat the wash step and remove the residual <b>RWB (RNA Wash Buffer)</b> as much as possible. Dry beads sufficiently ( 1-2 min)

## RELATED PRODUCTS

P-9003	Methylamp™ RNA Bisulfite Conversion Kit
P-9005	EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric)
P-9007	EpiNext™ 5-mC RNA Bisulfite-Seq Easy Kit (Illumina)
P-9008	EpiQuik™ m6A RNA Methylation Quantification Kit (Fluorometric)
P-9009	MethylFlash™ 5-mC RNA Methylation ELISA Easy Kit (Fluorometric)
P-9013	The EpiGenase™ m6A Demethylase Activity/Inhibition Assay Kit
P-9015	MethylFlash™ Urine N6-methyladenosine (m6A) Quantification Kit (Colorimetric)
P-9016	The EpiQuik™ CUT&RUN M6A-Seq Kit
P-9018	EpiQuik™ CUT&RUN M6A Enrichment Kit
P-9107	EpiQuik™ Viral RNA Isolation Fast Kit
P-9109	EpiMag™ 96-Well Viral RNA Extraction Kit (High Throughput)