

# EpiMag™ 96-Well Viral RNA Isolation Kit (High Throughput)

Base Catalog # P-9109

## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

**Uses:** The EpiMag™ 96-Well Viral RNA Isolation Kit (High Throughput) is suitable for a quick preparation of viral RNA in a high throughput manner 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 with the best volume of 135 µl. A total of 96 standard extractions can be performed with this kit.

**Binding Capacity and Yield:** The beads binding capacity can be up to 2 µg. RNA spiking tests show that the yield is >80%. 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	96 Samples P-9109-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
SRB (Sample Release Buffer)	30 ml	RT	RT	
10X RLB (RNA Lysis Buffer)	1.8 ml	RT	RT	
EB (Elution Buffer)	2 ml	RT	RT	
MD-Beads	18 ml	RT	4°C	
96-Well Assay Plate	1	RT	RT	

## SHIPPING & STORAGE

The kit is shipped at ambient room temperature. Upon receipt: Store the **MD-Beads** 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 (96-well microtube format), The EpiMag HT (96-Well) Magnetic Separator (#Q10002) from Epigentek is the best compatible to use of this kit.
- Pipettes, multi-channel pipette and RNase-free pipet tips
- 0.5 ml microcentrifuge tubes (RNase-free)
- 0.2 ml PCR tubes (RNase-free)
- 50 ml solution reservoir
- Ethanol (90%)

## GENERAL PRODUCT INFORMATION

**Quality Control:** Each lot of the EpiMag™ 96-Well Viral RNA Isolation Kit (High Throughput) 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™ 96-Well Viral RNA Isolation Kit (High Throughput) is for research use only and is not intended for diagnostic or therapeutic application.

## A BRIEF OVERVIEW

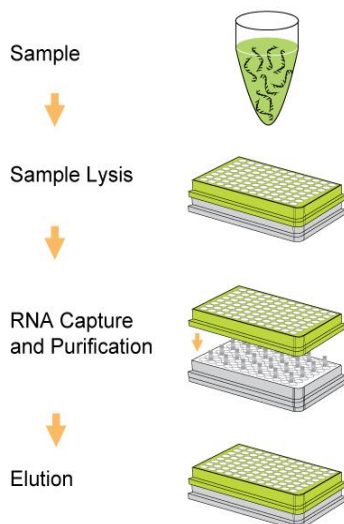
The EpiMag™ 96-Well Viral RNA Isolation Kit (High Throughput) provides a fast, simple, and cost-effective method for the isolation of viral RNA in a high throughput manner from cell-free liquid specimens (excluding plasma and serum), specifically from saliva and nasal or nasopharyngeal swabs. The specialized 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™ 96-Well Viral RNA Isolation Kit (High Throughput) is specifically suitable for RT-PCR.

The kit has the following features:

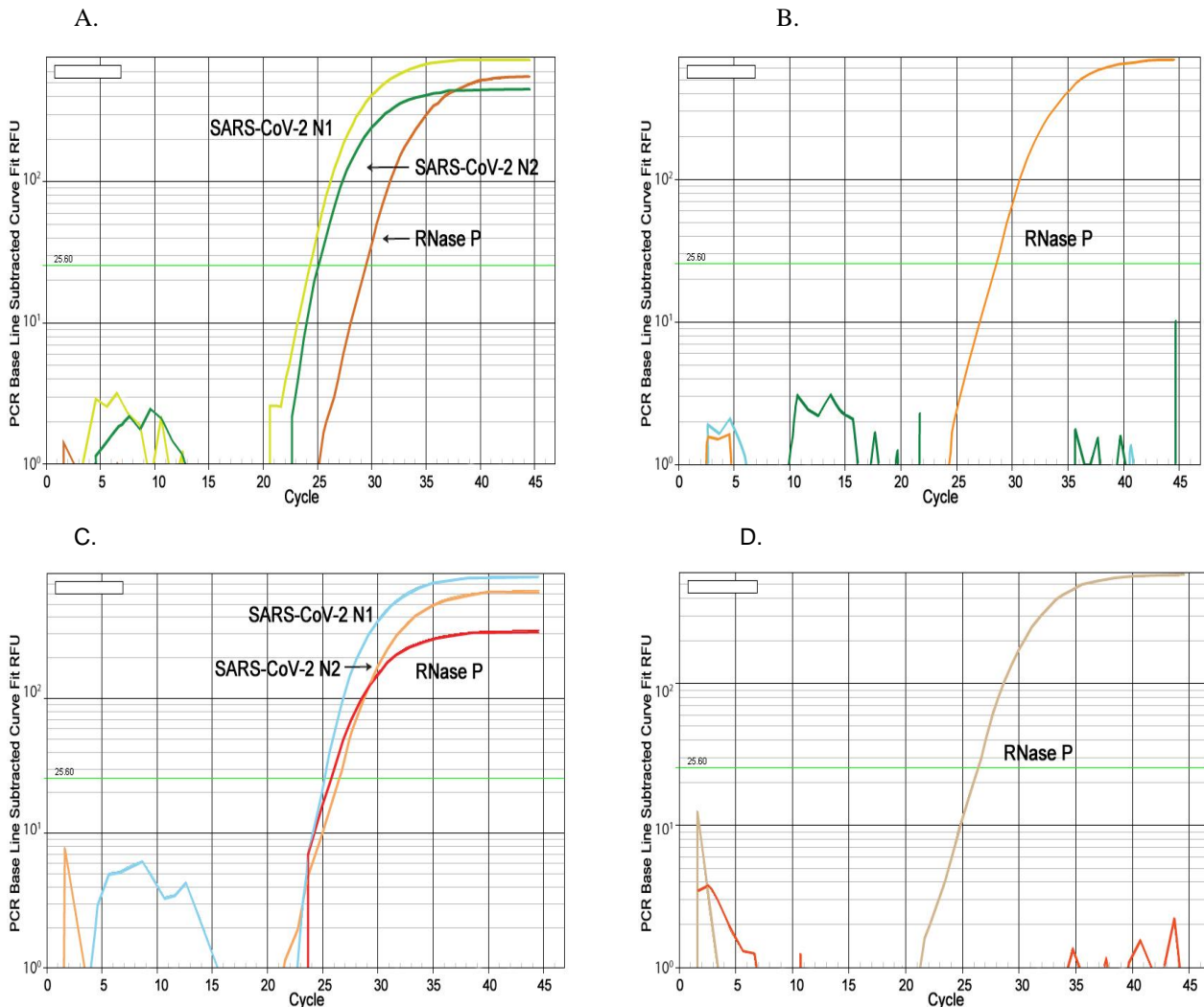
- High throughput and fast procedure allow to deliver total RNA from 96 samples simultaneously within 30 minutes
- Allowing small size (>200 bases) and large size (<200 kb) to be isolated
- Suitable for robot handling or automation setup without the need for centrifugation
- Ready-to-use RNA for high performance in downstream application
- Consistent RNA yield from a small amount of starting material

## PRINCIPLE & PROCEDURE

The EpiMag™ 96-Well Viral RNA Isolation Kit (High Throughput) 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 2 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™ 96-Well Viral RNA Isolation Kit (High Throughput).



**Fig 2.** Total RNA was isolated from nasal swab and saliva samples with EpiMag™ 96-Well Viral RNA Isolation Kit (High Throughput) and was used for RT-PCR analysis. A: nasal swab sample was spiked with SARS-CoV-2-N positive control; B: nasal swab sample was not spiked with SARS-CoV-2-N positive control. C: saliva sample was spiked with SARS-CoV-2-N positive control; D: Saliva 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, SARS-CoV-2 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 standard sample amount is 135 µl volume.

## 1. Sample Harvesting

### Liquid viral sample:

- 1.1a Directly collect 135  $\mu$ l of liquid samples into a well of the 96-well assay plate.
- 1.2a Add 15  $\mu$ l of the 10 X **RLB** (10 X RNA Lysis Buffer). Mix by pipetting.
- 1.3a Incubate at room temperature for 5 min and meanwhile re-suspend **MD-Beads** by shaking or vortexing.

### 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  $\mu$ l of **SRB** (Sample Release Buffer) and rotate the swab in SRB for 30 seconds.
- 1.3b Remove the swab and discard. Transfer 135  $\mu$ l of the sample into a well of the 96-well assay plate followed by adding 15  $\mu$ l of the 10X **RLB** (10 X RNA Lysis Buffer) to the sample. Mix by pipetting.
- 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. And 135  $\mu$ l of the transport media containing swab sample into a well of the 96-well assay plate and then add 15  $\mu$ l of the 10X **RLB** (10 X RNA Lysis Buffer) to the sample. Mix by pipetting.
- 1.5b Incubate at room temperature for 5 min and meanwhile re-suspend **MD-Beads** by shaking or vortexing.

### Saliva sample:

- 1.1c Collect 50  $\mu$ l of saliva sample into a well of the 96-well assay plate.
- 1.2c Add 85  $\mu$ l of **SRB** (Sample Release Buffer) followed by adding 15  $\mu$ l of the 10 X **RLB** (10 X RNA Lysis Buffer). Mix by pipetting.
- 1.3c Incubate at room temperature for 5 min and meanwhile re-suspend **MD-Beads** by shaking or vortexing.

## 2. RNA Isolation

- 2.1 After incubation, add 160  $\mu$ l of **MD-Beads**. Mix the sample by pipetting up and down at least 10 times, and then incubate at room temperature for 5 min.
- 2.2 Put the plates in the magnetic device until the solution is clear (about 8 min). Carefully remove and discard the supernatant.
- 2.3 Keep the plate in the magnetic device and add 300  $\mu$ l of 90% ethanol to the tubes, then carefully remove and discard the supernatant.
- 2.4 Repeat step 2.3 once for a total of two washes. Remove the ethanol as much as possible.

**Note:** (1) If saliva sample is used, pulling down MD-beads at step 2.2 may need 10-15 min for the solution to be clear; (2) If multiple samples or 96 samples are processed at the same time, a solution reservoir can be used for adding MD-beads with multi-channel pipette.

### 3. Elution

- 3.1 Keep the plate 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 plate 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 or PCR plate for immediate use or store at  $-20^{\circ}\text{C}$  for up to 2 months or at  $-80^{\circ}\text{C}$  for long term.

### 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 <b>MD-Beads</b> is added. And ensure 90% ethanol is properly prepared.
	Incorrect elution conditions	Add 20 $\mu$ l of the <b>EB (Elution Buffer)</b> to the beads, Incubate for at least 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 ethanol 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	Epigenase™ m6A Demethylase Activity/Inhibition Assay Kit
P-9015	MethylFlash™ Urine N6-methyladenosine (m6A) Quantification Kit (Colorimetric)
P-9016	EpiQuik™ CUT&RUN M6A-Seq Kit
P-9018	EpiQuik™ CUT&RUN M6A Enrichment Kit
P-9107	EpiQuik™ Viral RNA Isolation Fast Kit
P-9108	EpiMag Viral RNA Isolation Kit (Magnetic Beads)