Viral RNA-mini EXTRATION Kit

Introduction

The **MakGen Viral Extraction Kit** is designed for the rapid and reliable purification of viral RNA using a simple spin-column method. It is suitable for a wide range of clinical sample types, including whole blood, plasma, serum, urine, viral culture suspensions, saliva, and nasal or oral swabs.

The kit uses optimized reagents to break open viral particles and bind RNA to a specialized purification column. During the process, unwanted materials such as proteins, inhibitors, and cellular debris are removed through a series of wash steps. This ensures that the final RNA is clean and of high quality, making it suitable for downstream applications such as RT-PCR, real-time PCR (qPCR), and sequencing.

The MakGen Viral Extraction Kit has been developed through ongoing research and development to provide a dependable and easy-to-use solution for laboratory workflows. It is designed to perform consistently under standard laboratory conditions and to support routine diagnostic and research testing.

After extraction, the purified RNA can be used immediately or stored under recommended conditions for later analysis, maintaining its quality and integrity.

Catalogue Numbers:

Scan with your smart-phone camera to view the online protocol /video.

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INSTRUCTION MANUAL Ver.1.0.0 Revised on: 4/22/2025

RAPID-RNA™ Viral Kit	(50 prep)	(250 prep)	Cat No
Buffer KLS44 concentrate	20 ml	100 ml	1
Buffer K1 (wash buffer 1)	12 ml	60 ml (x2)	2
Buffer LS 2(Wash Buffer 2)	12 ml	60 ml (x2)	3
Elution buffer	10ml	50ml	4
Mak-Spin IC Columns	50	250	5
Collection Tubes	100	500	6
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Microfuge Tubes	50	100	8

Storage Temperature

Store all Makgen Viral Extraction Kit components (i.e., buffers, columns) at room temperature (15–25°C). When maintained under these conditions, the kit remains stable for at least 12months without any loss of performance, unless otherwise specified on the product label. If longer storage is required or if ambient temperatures often exceed 25°C, we recommend storage at 2–8°C.

Intended Use

The Makgen extraction Kit is designed for the **isolation and purification of nucleic acids (DNA and RNA)** from a variety of biological specimens including whole blood, plasma, serum, urine, viral culture suspensions, saliva, and nasal or oral swab samples.

The extracted nucleic acids are suitable for downstream molecular applications such as: PCR, RT-PCR, qPCR, Sequencing, Next-generation sequencing, Genotyping and Molecular diagnostics.

This kit is intended for **research use or in vitro diagnostic laboratory use** depending on regulatory classification.

Safety Information

When handling reagents and clinical samples, always adhere to standard laboratory safety procedures. Wear an appropriate lab coat, disposable gloves, and protective eyewear always. For detailed information on the safe handling, storage, and disposal of chemicals, refer to the relevant **Safety Data Sheets (SDSs)**.

All clinical specimens should be treated as potentially infectious and handled in accordance with applicable biosafety guidelines.

Before use:

- **Buffer Preparation**

1. **β-mercaptoethanol (β-ME)** has been pre-added to the lysis buffer where applicable. Handle with care and avoid inhalation or skin contact.
2. Add **16 mL of 100% ethanol** to **12 mL Buffer K1 concentrate**, or **80 mL of 100% ethanol** to **60 mL Buffer K1 concentrate**. Mix thoroughly before use.

Add **24 mL of 100% ethanol** to **12 mL Buffer LS2 concentrate**, or **120 mL of 100% ethanol** to **60 mL Buffer LS2 concentrate**. Mix thoroughly before use.

Ensure all reagents are prepared and labelled appropriately prior to starting the procedure.

TERMS

Sample Sources – ≤ 400 µl plasma, serum, saliva, swab, urine, blood, viral culture suspension.

For samples in UTM®/VTM®, PBS or saline, see Sample Preparation, page 8.

Purity – RNA is ready for downstream molecular work

Binding Capacity – 10 µg total RNA (**MAK-Spin™ IC Columns**).

Elution Volume – ≥ 6 µl **Elution buffer**.

Equipment Needed (user provided)

Microcentrifuge.

Materials (available separately) – Ethanol (100%),

Product Description

The ***rapid*-RNA™ Viral Kit** is a quick, purification of viral RNA from biological specimens.

The kit also features a buffer system that facilitates complete viral particle lysis for efficient nucleic acid isolation. Small (> 50 nt) and large (> 200 kb) RNA are bound to the column, washed, and eluted.

The isolated high-quality, total RNA is ready for all downstream applications such as Sequencing, Hybridization-based and PCR Assays.

Quality Control

Each lot of the Makgen viral RNA extraction Kit is tested against predetermined specifications to ensure consistent product quality.
To ensure extraction quality, laboratories should include both positive and negative extraction controls, measure nucleic acid concentration using a spectrophotometer or fluorometer, and confirm the suitability of the extracted nucleic acids for downstream applications by verifying amplification through PCR.

Principle and procedure

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For all protocols:

14.3 M b-mercaptoethanol (b-ME) (commercially available solutions are usually 14.3 M)

Sterile, RNase-free pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)

2 ml Safe-Lock microcentrifuge tubes (available from Brinkmann, cat. no. 022363352, or Eppendorf, cat. no. 0030 120.094), or 2 ml Safe Seal microcentrifuge tubes (Sarstedt, cat. no.72.695)

Microcentrifuge (with rotor for 2 ml tubes)

96–100% ethanol*

Isopropanol

Chloroform (for lipid-rich tissues e.g., fat, brain, breast)

Disposable gloves

Handling and storing starting material

Proper handling and storage of samples are critical to ensure the integrity of viral RNA and the reliability of downstream results.

General Handling

- Treat all specimens as potentially infectious and handle in accordance with applicable biosafety guidelines.
- Use appropriate PPE (lab coat, gloves, eye protection) always.
- Perform sample handling in a biosafety cabinet where applicable.
- Use RNase-free consumables and avoid repeated opening of sample tubes.
- Minimize sample processing time to reduce RNA degradation.

Sample Storage

Short-term storage:

- Store samples at 2–8°C for up to 72 hours prior to extraction.

Long-term storage:

- Store samples at −20°C to −80°C.
- For optimal RNA preservation, use −80°C whenever possible.
- Avoid repeated freeze–thaw cycles, as these can degrade viral RNA and reduce yield.

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) RNA Purification.

(I) Buffer Preparation

- ✔ Already contains beta-mercaptoethanol
- ✔ Add 16 ml of 100% ethanol to the 12 ml **Buffer K1** concentrate
- ✔ Add 24ml of 100% ethanol to the 12 ml **Buffer LS2** concentrate

(II) Sample Preparation

- ✔ Perform all steps at room temperature (20-30°C).
- ✔ Up to 400 µl of sample can be processed per prep.

Samples in collection devices (swabs, saliva, etc.) Proceed directly with purification, page 10

Swabs (UTM®/VTM®, PBS, saline, etc.)

Proceed directly with purification, page 10

Optional - To inactivate, store and preserve samples at room temperature prior to further processing,

Optional - **Proteinase K treatment** (protein-rich samples e.g., plasma, serum, saliva, sputum, tissue, can be treated).

Add 1% **Proteinase K** (v/v) at 20 mg/ml directly to a liquid sample. Mix well and incubate at room temperature for 15 minutes.

Note: Up to 5% Proteinase K can be added (e.g., tissue).

For example: Add 4-20µl Proteinase K to each 400µl sample.

(III) RNA Purification

✔ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g.

✔The sample input can be scaled up or down, proportionally.

1. Transfer 100ul of the sample into a 1.5ml microcentrifuge tube Add 280ul of the KLS44 Lysis buffer into 1.5ml microcentrifuge tube, the sample, vortex and incubate at room temperature for 10 minutes.

2. Add 280 ul of ethanol (100 %) to each sample, transfer the mixture into spin columns and centrifuge at 14000 rpm for 2 minutes.

3. Place the spin columns in new clean collection tubes and add 300ul of the K1 Wash Buffer 1(WB 1) and centrifuged at 14000 rpm for 2 minutes.

4. Place the spin columns into new collection tubes, add 300ul of LS2 Wash Buffer 2(WB 2) and centrifuge at 14000 rpm for 5 minutes.

5. Place the spin columns in new corresponding 1.5 ml microcentrifuge tubes, add 15 ul of Elution Buffer into each tube and centrifuged at 14000 rpm for 1 minute to elute the RNA.

The eluted RNA can be used immediately or stored frozen.

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
RNA degradation	To prevent RNA degradation: Immediately collect and lyse fresh samples into a stabilization reagent (i.e., RNA Shield) to ensure nucleic acid stability. Homogenized samples in RNA Shield can be stored frozen for later processing.
Low nucleic acid content and/or low sensitivity in downstream application	Incomplete deproteinization due to high-protein content in the sample (blood, plasma/serum, tissue etc.): - Increase the volume of RNA Shield to the sample. - Perform Proteinase K treatment (see Sample Preparation, page 4). Increase eluate input: -Titrate the RNA eluate for downstream applications (i.e., RT/qPCR).
DNA contamination	To remove DNA: - Perform DNase I treatment during the purification (page 6) or perform DNase I treatment post-purification (#R1013), then clean-up the treated sample.

General Remarks on Handling RNA

Storage of RNA Purified RNA may be stored at –30°C to –15°C or –90°C to –65°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

NOTES

Limitations

- Extraction efficiency may vary depending on the type, source, and condition of the sample.
- Highly viscous specimens may require pre-treatment or dilution to enable effective processing.
- The overall sensitivity and reliability of downstream assays are dependent on the quality and integrity of the extracted RNA.
- Poor-quality, degraded, or improperly stored samples may result in low RNA yield **or** false-negative results.
- Samples with low viral load may yield insufficient RNA for detection, even when extraction is performed correctly.
- Highly viscous samples (e.g., saliva or sputum-like material) should be properly homogenized or diluted to avoid column clogging and reduced yield.
- Incomplete removal of wash buffer may result in ethanol carryover, which can inhibit RT-PCR or qPCR reactions.

- Variations in pipetting accuracy, mixing, or centrifugation conditions may affect performance and reproducibility.
- Failure to detect the internal control may indicate extraction inefficiency or assay inhibition, and results should be interpreted with caution.
- Improper handling or workspace contamination may lead to false-positive results**.** Use aerosol-resistant tips and maintain workflow separation.

Performance Characteristics

Parameter	**Performance**
Extraction time	25–40 minutes
Yield	1–50 µg depending on sample
RNA integrity	Suitable for RT-PCR, PCR and sequencing

Disposal

Dispose of used reagents and biological materials according to:

- Institutional biosafety guidelines
- National biomedical waste regulations

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MaK-BRC Diagnostic unit on +256784530401.

Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research and Diagnostic purposes and should only be used by trained professionals.

Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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