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User Guide

Ultra pure MagPrep® Viral RNA Isolation Kit

Isolation of viral nucleic acid from biological fluids (Cat. No. UPMPVRNA-KT)

Introduction

Ultra pure DNA-free MagPrep® Viral RNA Isolation Kit can be utilized for manual or high throughput nucleic acid isolation from biological fluids. Buffers have been designed to maximize the performance of the magnetized silica particles and this rapid and simple protocol describes manual isolation of nucleic acid. Buffer components are compatible with automation systems. The user-friendly protocol has no Proteinase K or heating steps, and all items are shipped and stored at ambient temperature.

All kit components (MagPrep® particles and buffers) are tested to be Ultra pure i.e. free of any Nickase, DNase, RNase, and DNA-free (tested by qPCR for 16S-, 18S-RNA genes and plasmid Ori). The DNA-free Ultra pure feature of the kit enables reliable results in downstream applications without the fear of false positives (DNA-free) and false negatives (nuclease-free). The purified nucleic acid is free of any PCR inhibitors (chaotropic agents, ethanol, etc.) and can be used for various downstream applications such as RT-qPCR, ddPCR, etc.

For research and further manufacturing use only.

Principle

MagPrep® magnetic particles are an easy and fast method to isolate nucleic acids. The magnetized silica particles are incubated with a matrix containing nucleic acid and MagPrep® Viral RNA Lysis and Binding Buffer. Nucleic acid binds to the particles and unwanted materials are washed away using a magnet. The isolate nucleic acid is then eluted using MagPrep® Elution Buffer. The process can be automated or performed manually in less than 30 minutes and yields high recovery of pure nucleic acid without any PCR inhibitors (see **Figure 1**).

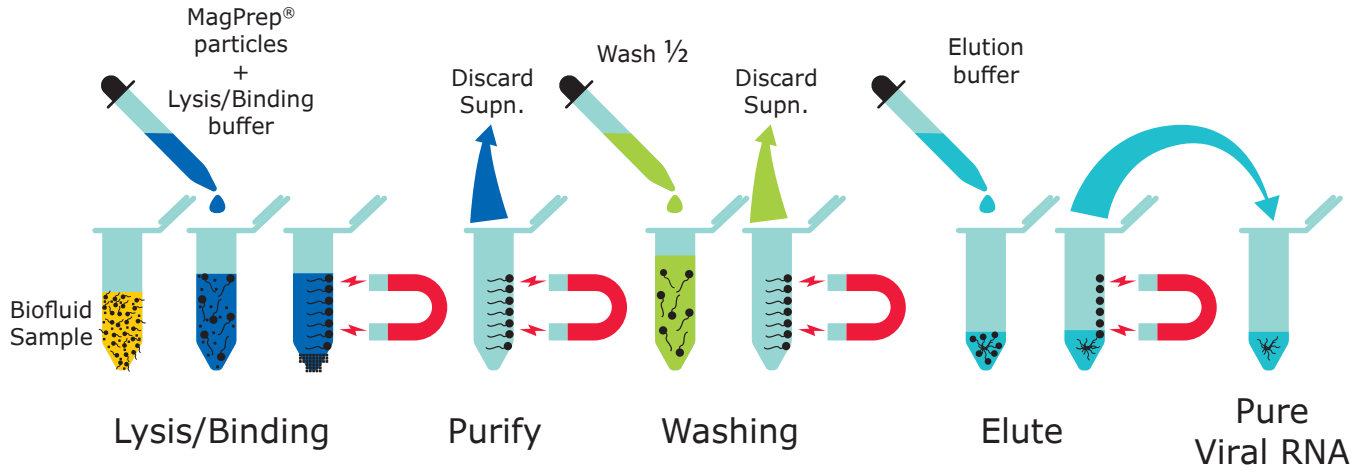

Figure 1: Graphical image of the process to create Ultra Pure Viral RNA.

Storage Conditions

Store kit components at ambient temperature.

DO NOT FREEZE.

Reagents Supplied:

100 reactions or one 96-well plate

Ultra pure MagPrep® Viral RNA Isolation Kit,
Cat. No. UPMPVRNA-KT

Kit Component Name	Cat. No.	Volume	Quantity
Ultra pure MagPrep® Viral RNA Lysis and Binding Buffer	**UPMPVRBB-KC**	40 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 1	**UPMPWB1-KC**	60 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 2	**UPMPWB2-KC**	60 mL	1 bottle
Ultra pure MagPrep® Elution Buffer	**UPMPEB-KC**	10 mL	1 bottle
Ultra pure MagPrep® Magnetic Particles	**UPMPPART-KC**	2 mL	1 vial

Individual Components available separately:

Component Name	Cat. No.	Volume	Quantity
Ultra pure MagPrep® Viral RNA Lysis and Binding Buffer	**UPMPVRBB**	200 mL, 400 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 1	**UPMPWB1**	300 mL, 500 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 2	**UPMPWB2**	300 mL, 500 mL	1 bottle
Ultra pure MagPrep® Elution Buffer	**UPMPEB**	100 mL, 250 mL	1 bottle
Ultra pure MagPrep® Magnetic Particles	**UPMPPART**	10 mL, 20 mL	1 vial

Materials Required (not included)

Reagents are needed for downstream applications (e.g. qPCR, ddPCR, NGS, Bisulfite-SEQ, etc.); review your application protocol to determine which reagents are needed.

Instrumentation/Materials

- Magnetic rack
- Rotating mixer
- Adjustable Pipettes with tips capable of delivering 25 µL to 1000 µL
- Polypropylene Microfuge Tubes
- Laboratory Vortex Mixer
- Benchtop microfuge

Safety Precautions

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Isopropanol and ethanol have been added to some of the buffers and must be treated as flammables. Other ingredients may have low amounts of corrosive materials as well as materials toxic to aquatic life. Dispose of unused contents and waste following international, federal, state, and local regulations.

Technical Guidelines

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

For research and further manufacturing use only. Not for use in diagnostic procedures.

Do not use beyond the expiration date on the label.

Do not mix or substitute reagents with those from other lots or sources.

The Lysis/Binding buffer is light-sensitive. It is packaged in a light protective bottle and must be protected from light if aliquots are taken from the original bottle.

Incomplete washing can adversely affect isolation purity. All washing must be performed with the Wash Buffers provided.

Be aware that nucleic acid can be sensitive to degradation at room temperature. Ensure isolation using this kit is conducted in a timely matter.

After the second wash, ensure complete removal of Wash Buffer 2.

Ensure components are recapped right after each use.

Lysis/Binding buffer may change to a yellowish color. This does not affect performance.

When using frozen samples, it is recommended to thaw the samples completely and mix them well by vortexing and centrifuging before use in the assay to remove particulates.

Fresh centrifuged and filtered plasma gives better recovery.

Nucleic Acid Isolation Procedure

(*Applicable to any biological fluid sample***)**

Manual Protocol

NOTE: Protocol may be optimized for customer need.

Scale: Protocol is written for 0.1–0.4 mL biological fluid as starting material that should be mixed with 0.4 mL of Viral RNA Lysis and binding buffer containing ≈0.8 mg MagPrep® particles for each binding reaction (step 1). The protocol is scalable.

Gently shake the buffers before use. Resuspend the MagPrep® particles stock solution thoroughly by vortexing before taking an aliquot.

1. In a 1.5 mL nuclease-free microfuge tube, add ~14 µL of Ultra pure MagPrep® particles to 400 µL of Ultra pure Viral RNA Lysis and Binding Buffer and mix well by pipetting up-down or vortexing.
2. Add ~100–400 µL of biological fluid sample and mix well by pipetting up and down 10–15x or vortexing intermittently 10 times at a medium speed.
3. Incubate for 5 minutes to allow nucleic acid binding (Optional: a rotation system to mix during incubation may be used).
4. Place the tube on a magnetic rack for 1–2 minutes to capture the nucleic acid-particle complexes, then discard the supernatant.
5. Remove the tube from the magnetic rack and resuspend the nucleic acid-particle complexes in 1 mL of Ultra pure Wash Buffer 1 by vortexing. Use a microfuge to spin and bring all contents down quickly.
6. Return to the magnetic rack for 1–2 minutes, then discard the supernatant.
7. Repeat wash with 1 mL of Ultra pure Wash Buffer 2. Use a microfuge to spin and bring all contents down quickly.
8. Return to the magnetic rack for 1–2 minutes, then discard the supernatant.
9. *Essential*: Do a quick spin on a microfuge to collect remnants of wash buffer sticking to the sides of the tubes and particles and return to the magnetic rack. Remove any remainder supernatant.
10. Dry the tube with the lid open at room temperature for 5 minutes. Dry for longer if pellet still looks wet.
11. After the ~5 minute drying step, remove any visible supernatant.
12. Remove the tube from the magnetic rack and resuspend the particles in ~50–100 µL of Ultra pure Elution Buffer.
13. To elute nucleic from particles, mix by pipetting 15–20x or vortexing intermittently 10 times at a medium speed.
14. Let stand for 1–2 minutes. Do a quick spin in a microfuge to bring all contents down.
15. Place the tube on a magnetic rack for 1–2 minutes to separate particles from the suspension.
16. Transfer the supernatant containing the isolated nucleic acid solution to a nuclease-free tube.
17. Store at –80 °C for long-term storage.

Troubleshooting Guide

Problem	Probable Cause	Solution
Poor recovery	Insufficient magnetic particles were aliquoted from the MagPrep® particles stock solution	Ensure the MagPrep® particles stock solution was mixed thoroughly by vortexing before taking an aliquot.
	Very dilute sample	Increase sample volume and/or increase particle binding incubation time.
	Lysis and binding buffer was skipped and MagPrep® particles were added directly to the plasma sample	Dilute MagPrep® particles in Lysis and Binding buffer before adding them to sample. Binding buffer removes proteins and facilitates nucleic acid binding to particles.
Inhibition of PCR	The sample has too much debris	Perform 1–2 additional washes with Wash Buffer 2
	The sample contains residual alcohol from Wash Solution 2	Post removal of wash solution 2 supernatant, remove the vial from the magnetic rack and spin briefly in a microfuge. Place back on a magnetic rack for 2 minutes and use a P20 pipette to remove any supernatant left in the vial.
	The sample contains magnetic particles	Avoid disturbing the magnetic particles while removing the supernatant. Angle the pipette so that the tip does not touch the magnetic particles. If particles are still observed in the final eluate they can be removed by another short magnetic separation on the rack.
Particles are not migrating to the magnet	The sample is too viscous	Dilute the sample or increase the time on a magnetic rack to >5 minutes.

Ordering Information

To place an order or to obtain additional information about our MagPrep® particles, buffers, and nucleic acid isolation kits, order online at **SigmaAldrich.com**.

For interest in bulk or custom SKUs, contact your local Account Manager or place a request through our website.

Conditions of Sale

For research and further manufacturing use only. Not for use in diagnostic procedures.

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