

Viral RNA Extraction Miniprep Kit

from Plasma, CSF, Urine, Other cell-free body fluids
and Cell culture supernatants



User Guide

Pack of 100 preps

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mdi
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1. Introduction

The **mdi** Viral RNA Extraction Miniprep Kit is a fast, economical and easy isolation method of high purity viral RNA from plasma, CSF, urine, other cell-free body fluids and cell culture supernatants. It has a silica membrane column which along with the buffer system provided in the kit allows efficient lysis followed by selective binding of RNA to the spin column and elution of purified viral RNA using centrifugation.

Purified RNA is eluted in low-salt buffer or water for variety of downstream applications. This technology does away with the cumbersome methodologies of phenol extraction (associated with slurries formation) as well as ethanol precipitation (associated with anion exchange based purification system) for desalting.

2. Applications

Purified Viral RNA is suitable for

1. RT-PCR, qRT-PCR, q-PCR
2. Viral genotyping
3. Viral Detection
4. Viral load monitoring
5. Viral epidemiology

3. Storage Conditions

Optimum storage conditions at which all components of the kit can be stored without alteration in its quality and performance.

One Year	15-25°C
Longer Storage	2-8°C

Important

In case of any precipitation observed in the buffers, re-dissolve by warming to 50°C for 10-15 minutes till it completely dissolves, and cool it down to room temperature.

4. Quality Assurance

The **mdi** Viral RNA Extraction Miniprep Kit is designed for various pre-determined specifications and user requirements such as yield, purity, ruggedness, shelf life and functional convenience.

These are produced through a well defined quality management system in clean GMP facilities which ensures intra lot as well as lot to lot consistency.

5. Safety Information

The buffers and the reagents may contain irritants, so wear lab coat, disposable gloves and protective goggles while working with the Viral RNA Extraction Miniprep Kit.

6. Lot Release Criteria

Each lot of Viral RNA Extraction Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

7. Technical Support

At **mdi**, customers are our priority. We will share our experiences to assist you to overcome problems in general product usage as well as customize products for special applications. We will

- * Simulate problems, and suggest alternative methods to solve them.
- * Make changes/improvements in our existing products/ protocols.
- * Develop special new products and systems especially to satisfy your needs.

**We welcome your feedback to improve our products.
Please write to info@mdimembrane.com**

8. Kit Contents

Contents	Quantity	Storage Temperature
Spin Columns	100	RT
Collection Tubes	100	RT
Buffer VRL	62ml	RT
Buffer VW1	31ml	RT
Buffer VW2	28ml	RT
Buffer VE	10ml	RT
Carrier RNA*	620µg	RT
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***NOTE: After reconstitution of carrier RNA (refer page 10), store at -20°C.**

9. Viral RNA Isolation Principle

Obtaining highly pure viral RNA using **mdi** Viral RNA Extraction Miniprep Kit involves:

Lysis of Sample

For efficient lysis, mix sample completely with buffer VRL, then incubate at room temperature for 10 minutes.

Capturing of viral RNA on Spin Column

In order to facilitate adsorption of viral RNA onto the spin column, optimum conditions of salt concentration and pH are required, which is achieved by addition of binding buffer VRL.

Washing

Subsequent to RNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away.

Washing is performed with buffers VW1 and VW2.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency. Elution occurs at basic conditions and low salt concentration. Elution is performed with buffer 'VE'. For obtaining highly concentrated viral RNA, perform elution with 40µl of buffer VE.

10. Procedure

Sample



Lyse



Bind



Wash 3X



Dry



Elute



Ultra Pure RNA

11. Specifications

Specifications	Range	Optimum Value
Volume of Sample	25-300µl	140µl
Capacity of column reservoir	up to 700µl	700µl
Binding capacity of membrane	up to 10µg	10µg
Elution volume	30µl-100µl	40µl
Time Taken	< 20 minutes	< 20 minutes

12. How to Begin

A. Items required by the user but not provided in the kit:

- 1.5 ml microcentrifuge tubes
- Sterile, RNase-free pipette tips (pipette tips with aerosol barriers for preventing cross contamination are recommended)
- Ethanol (96–100%)*
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- Vortex Mixer

B. Sample collection and storage

Do's: For obtaining best results, the starting material should be either fresh or that has been immediately frozen and stored at -20 °C or -70 °C.

Don'ts: Poor-quality starting material leads to reduced length and yield of purified RNA.

C. Maximum Amount of Starting Material

Do's: Optimum starting sample should be 140µl.

Don'ts: Overloading of **mdi** spin columns leads to significantly lower yields than expected.

D. Elution

With **mdi** Viral RNA Extraction Miniprep Kit, highly concentrated RNA is obtained with 40µl of buffer VE.

* Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.

12.1 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Optimum amount of sample should be 140 μ l.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature at $\geq 10,000$ rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. Check buffer VRL. In case of precipitation, re-dissolve by warming to 50°C for few minutes.
2. For efficient lysis, mix sample completely with buffer VRL, then incubate at room temperature.

Washing

1. To remove residual wash buffer, spin the column with closed lid for 1 minute at $\geq 10,000$ rpm.

Elution

1. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 5-6 minutes.
2. For obtaining highly concentrated RNA, perform elution with 40 μ l of buffer VE.

12.2 Protocol

Note: Things to do before starting

1. Arrange ethanol (96-100%).
2. Add recommended volume of **Ethanol (96-100%)** to buffer VW1 and buffer VW2 before use as shown in Table below.

Buffer	Buffer Volume	Volume of Ethanol(96-100%) to be added
VW1	31ml	27ml
VW2	28ml	72ml

3. Check buffer VRL. If precipitates are observed, incubate at 50°C in original bottle for few minutes until they redissolve. Cool buffer back to room temperature before use.
4. Dissolve carrier RNA completely in buffer VE by adding 620µl of buffer VE to 620µg of carrier RNA contained in tube provided to make stock solution of 1µg/µl. Prepare convenient sized aliquots and store at -20°C. Avoid repeated freeze-thaw cycles more than three times.
5. **Addition of Carrier RNA to Buffer VRL:** For every test to be conducted, 5.6µl of carrier RNA stock solution of 1µg/µl from step 4 above needs to be added to 560µl of buffer VRL. **Buffer VRL-carrier RNA solution** should be prepared fresh, but for convenience, if the frequency of testing/use of kit reactions is high, the 620µl of carrier RNA stock solution from step 4 above may be fully added to bottle containing 62ml of buffer VRL. **Buffer VRL-carrier RNA solution** is stable for up to 48 hours if stored at 2-8°C and would be good for more than 100 tests.

Note: If precipitation is observed after storing the **Buffer VRL-carrier RNA solution** at 2-8°C, incubate for 10-15 minutes until it redissolves. Frequent warming (more than 6 times) and long incubation may cause degradation of carrier RNA and should be avoided.

6. Use RNase free plastics and glasswares.

Procedure:

1. Pipet 560µl of **Buffer VRL-carrier RNA solution** into a 1.5ml microcentrifuge tube (not provided).
2. Add 140µl sample to the buffer VRL-carrier RNA in the microcentrifuge tube. Mix by vortexing for 15 seconds.

Note: For efficient lysis, it is essential that the sample is mixed thoroughly with buffer VRL-carrier RNA solution to yield a homogeneous solution. The protocol is optimized for 140µl sample volumes, but sample volumes 25-300µl can also be processed. For samples larger than 140µl, the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally. The volume of wash buffers VW1 and VW2 is not required to be increased. The volumes of samples less than 140µl should be adjusted to 140µl with phosphate buffered saline (PBS) and low viral titre should be concentrated to 140µl before processing.

3. Incubate at room temperature for 10 minutes.
4. Briefly centrifuge the tube to remove drops from inside of the lid.
5. Add 700µl of Ethanol (96-100%) to the above sample mixture and vortex. After vortexing, briefly centrifuge the tube to remove drops from inside the lid.

For efficient binding, it is essential that mixing with ethanol is thorough to yield a homogeneous solution.

6. Place the spin column in the collection tube. Pass the above 700µl solution from step 5 through the spin column by spinning at $\geq 10,000$ rpm for 1 minute. Discard the flow through but save the collection tube.
7. Repeat step 6 with the remaining solution. Starting sample volume greater than 140µl will require multiple repetition of step 6.

Note: If the sample does not pass after 1 minute then centrifuge for additional 1 minute.

8. Place the spin column in the same collection tube. Wash the column with 500µl of buffer VW1 by centrifuging for 1 minute at $\geq 10,000$ rpm. Discard the flow through but save the collection tube.
9. Place the spin column in the same collection tube. Wash the column with 500µl of buffer VW2 by centrifuging for 1 minute at $\geq 10,000$ rpm. Discard the flow through but save the collection tube.
10. Place the spin column in the same collection tube. Wash the column with 400µl of buffer VW2 by centrifuging for 2 minutes at $\geq 10,000$ rpm. Discard the flowthrough and collection tube.
11. Place the spin column in a RNase free 1.5ml microcentrifuge tube (not provided). Elute the bound viral RNA by adding 40µl buffer VE directly to the center of the column membrane and let it stand for **5 minutes**. Spin at $\geq 10,000$ rpm for 1 minute.

User can elute viral RNA in 30-100µl but optimum elution volume is 40µl. Performing double elution using 2x40µl or 2x50µl can increase total RNA yield slightly.

Note: Store RNA at -20°C to -80°C.

13. Trouble Shooting Guide

A. Little or no Yield of Viral RNA

- | | |
|-----------------------------------|--|
| 1. Overloading of spin column | Can happen if larger volume of sample is taken than recommended. |
| 2. Precipitates in buffer | In case of any precipitates in buffers, re- dissolve by warming to 50°C in original bottle before use. |
| 3. Low percentage of ethanol used | Use 96-100% ethanol wherever required. Do not use denatured alcohol or low percentage of ethanol |
| 4. Spin column choked | Increase centrifugation time while passing sample through the spin column. |

- | | |
|---|--|
| 5. Insufficient Lysis | Increase incubation time. Increase incubation temperature. |
| 6. Improper dispensing of elution buffer | The elution buffer must be dispensed properly on to the center of the column membrane. |
| 7. Insufficient incubation of elution buffer in the column membrane | Increase incubation time by 2-3 minutes. |
| 8. Insufficient mixing of sample with buffer VRL and ethanol before loading on to the spin column | Add buffer VRL to the sample first and mix by vortexing. After incubation, add ethanol to the sample and again mix by vortexing. |
| 9. Poor Quality Sample | Use fresh sample or sample stored at -20°C to -70°C. Avoid repeated freezing and thawing of sample. |
| 10. Improper storage of samples | For short term storage, samples should be stored at 4°C in tubes. For long term, store at -20°C to -70°C. |

B: Low quality RNA

- | | |
|---------------------------|---|
| 1. Nuclease contamination | Use RNase free plastics and glasswares. |
| 2. Sheared RNA | Avoid vigorous mixing. |

C: RNA does not perform well

- | | |
|-----------------------------------|---|
| 1. Residual wash buffer in eluate | After second washing with VW2, discard the flowthrough and place the spin column in the same collection tube with closed lid. Centrifuge for additional 2-3 minutes at $\geq 10,000$ rpm. |
|-----------------------------------|---|

2. Centrifugation less than 10,000rpm in drying step. Centrifuge at $\geq 10,000$ rpm in drying step. If centrifuge rpm is less than 10,000rpm then discard the flowthrough after second washing with VW2, and place the spin column in the same collection tube with closed lid. Centrifuge for additional 2-3 minutes.

14. Product Use Limitations

mdi kits are developed and manufactured for research purpose only. The products are not recommended to be used for human, diagnostics or drug purposes for which these should be cleared by the concerned regulatory bodies in the country of use.

15. Product Warranty and Satisfaction Guarantee

All **mdi** products are guaranteed and are backed by our

- a. Technical expertise and experience of over 40 years.
- b. Special **mdi** process for consistency and repeatability.
- c. Strict quality control and quality assurance regimen.
- d. Certificate of Quality.

mdi provides an unconditional guarantee to replace the kit if it does not perform for any reasons other than misuse. However, the user needs to validate the performance of the kit for its specific use.

16. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
VMRK	VMRK					100	0100
						250	0250

Example:

VMRK	XX	XX	XX	X	0100
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Manufacturing Site

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