Viral RNA Extraction Miniprep Kit

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

User Guide

Membrane Technologies

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Table of Contents

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 preserved without alteration in it's quality and performance.

One Year	RT
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 minute 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 Temp.
Spin Columns	10	25	50	100	300	RT
Collection Tubes	10	25	50	100	300	RT
Buffer VRL	7ml	17.5ml	35ml	70ml	105ml x 2	RT
Buffer VW1	3.1ml	7.75ml	15.5ml	31ml	31ml x 3	RT
Buffer VW2	2.8ml	7ml	7ml X 2	28ml	28ml x 3	RT
Buffer VE	5ml	5ml	10ml	10ml	30ml	RT
Carrier RNA*	70µg	175µg	350µg	700µg	2100µg	-20°C
Hand Book	1	1	1	1	1	–
COQ	1	1	1	1	1	–

***Note:**

1. **Reconstitute carrier RNA in buffer VE .**
2. **Add recommended volume of Ethanol (96-100%) to buffer VW1 and buffer VW2 before use.**

Refer page 10.

9. Specifications

Volume of Sample	140µl
Capacity of column reservoir	700µl
Binding capacity of membrane	10µg
Elution volume	40µl
Time Taken	< 20 minutes

10. How to Begin

A. 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.

B. Maximum Amount of Starting Material

Do's

Maximum starting sample should be 140µl.

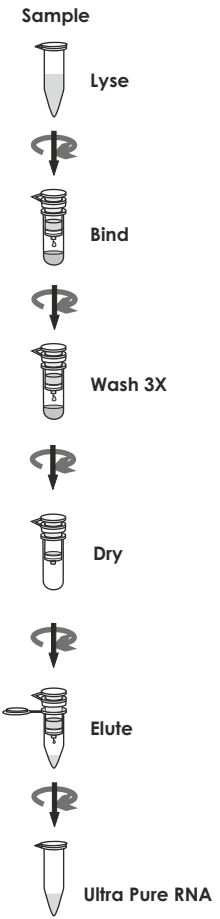
Don'ts

Overloading of **mdi** spin columns leads to significantly lower yields than expected.

C. Elution

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

11. Procedure



12. Viral RNA Isolation

12.1 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.

12.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of sample should be 140µl.

Centrifugation:

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

Lysis

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

Washing

1. To remove residual wash buffer, spin the column with closed lid for 1 minute at ≥ 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 40µl of buffer VE.

12.3 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 1.
3. Reconstitute carrier RNA in buffer VE to prepare stock 1µg/µl and store at -20°C.

Carrier RNA	70µg	175µg	350µg	700µg	2100µg
Buffer VE	70µl	175µl	350µl	700µl	2100µl

4. If precipitates are observed in Buffer VRL, incubate at 50°C for few minutes.
5. Add 5.6µl carrier RNA to 560µl Buffer VRL to give concentration (5.6µg/560µl).
6. Use RNase free plastics and glasswares.

Table1:

Spin column pack	Buffer	Buffer Volume	Volume of Ethanol(96-100%) to be added
10	VW1	3.1ml	2.7ml
25	VW1	7.75ml	6.75ml
50	VW1	15.5ml	13.5ml
100	VW1	31ml	27ml
300	VW1	31ml x 3	27ml x 3

Spin column pack	Buffer	Buffer Volume	Volume of Ethanol(96-100%) to be added
10	VW2	2.8ml	7.2ml
25	VW2	7ml	18ml
50	VW2	7ml x 2	18ml x 2
100	VW2	28ml	72ml
300	VW2	28ml x 3	72ml x 3

Procedure:

1. Pipet 560µl of prepared buffer VRL containing 5.6µg carrier RNA into a microcentrifuge tube.

2. Add 140µl sample to the buffer VRL-carrier RNA in the 1.5ml microcentrifuge tube. Mix by vortexing for 15 seconds.

   Note: For efficient lysis, it is essential that the sample is mixed thoroughly with buffer VRL to yield a homogeneous solution.

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 sample and mix by vortexing. After mixing, briefly centrifuge the tube to remove drops from inside the lid.

   For efficient binding, it is essential that the sample is mixed thoroughly with ethanol to yield a homogeneous solution.

6. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at $\geq$ 10,000 rpm for 1 minute. Discard the flow through.

   Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample and spin at $\geq$ 10,000 rpm for 1 minute. Discard the flow through. If the sample does not pass after 1 minute then centrifuge for additional 1 minute.

7. Place the spin column in the same collection tube. Wash the column with 500µl of buffer VW1 by centrifuging for 1 minute at $\geq$ 10,000rpm. Discard the flowthrough.

8. Place the spin column in the same collection tube. Wash the column with 500µl of buffer VW2 by centrifuging for 1 minute at $\geq$ 10,000rpm. Discard the flowthrough.

9. Place the spin column in the same collection tube. Wash the column with 400µl of buffer VW2 by centrifuging for 2 minute at ≥ 10,000rpm. Discard the flowthrough, and collection tube.

10. 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 ≥ 10,000rpm for 1 minute.

    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 before use.
3. Spin column choked	Increase centrifugation time while passing sample through the spin column.
4. Insufficient Lysis	Increase incubation time. Increase incubation temperature.
5. Improper dispensing of elution buffer	The elution buffer must be dispensed properly on to the center of the column membrane.
6. Insufficient incubation of elution buffer in the column membrane	Increase incubation time by 2-3 minutes.

7. 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.
8. Poor Quality Sample	Use fresh sample or sample stored at -20°C t o -70°C. Avoid repeated freezing and thawing of sample.
9. 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.
3. Sheared RNA	Avoid vigorous mixing.

C: RNA does not perform well

Residual wash buffer in eluate	After 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.

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 analysis accompanied with each product.

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
Type	Code				
VMRK	VMRK				

Pack Size	
Pack Size	Code
50	0050
100	0100
300	0300

Example:

VMRK	XX	XX	XX	X	0050

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