Viral RNA/DNA Miniprep Kit

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

User Guide

mdi
Membrane Technologies

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1. Introduction

The **mdi** Viral RNA/DNA Miniprep Kit is a fast, economical and easy isolation method of high purity viral RNA/DNA 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/DNA to the spin column and elution of purified viral RNA/DNA using centrifugation.

Purified RNA/DNA 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/DNA is suitable for

1. RT-PCR, qRT-PCR, q-PCR, 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 it's 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 56°C for 10-15 minutes till it completely dissolves, and cool it down to room temperature.

4. Quality Assurance

The **mdi** Viral RNA/DNA 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/DNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Viral RNA/DNA Miniprep Kit is tested against pre-determined 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	50	RT
Collection Tubes	50	RT
Buffer RDL	31ml	RT
Buffer RDW1	15.5ml	RT
Buffer RDW2	14ml	RT
Buffer RDE	10ml	RT
Proteinase K	32mg	2-8°C
Proteinase K Buffer	1.6ml	2-8°C
Carrier RNA	310µg	RT
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NOTE:

1. After reconstitution of carrier RNA (refer page 10), store at - 20°C.
2. Reconstitute Proteinase K (32mg) in proteinase K buffer (1.6ml) (provided) to prepare stock of 20mg/ml.
3. Add recommended volume of Ethanol (96-100%)to buffer RDW1 and buffer RDW2 before use(See page 10).

9. Viral RNA/DNA Isolation Principle

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

Lysis of Sample

For efficient lysis, sample is mixed with Proteinase K and buffer RDL, and incubate at 56°C.

Capturing of viral RNA/DNA on Spin Column

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

Washing

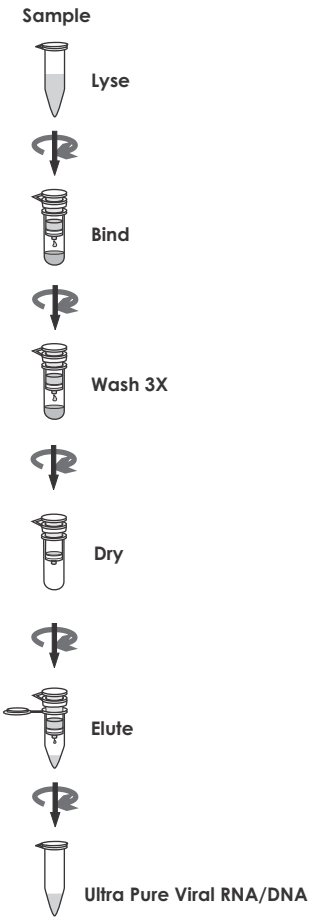
Subsequent to RNA/DNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away.

Washing is performed with buffers RDW1 and RDW2.

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 'RDE'. For obtaining highly concentrated viral RNA/DNA, perform elution with 40µl of buffer RDE.

10. Procedure



11. Specifications

Specifications	Optimum Value
Volume of Sample	200µl
Capacity of column reservoir	700µl
Binding capacity of membrane	10µg
Elution volume	40µl

12. How to Begin

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

- 1.5 ml microcentrifuge tubes
- Sterile, RNase/DNase 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/DNA.

C. Maximum Amount of Starting Material

Do's: Optimum starting sample should be 200µl.

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

D. Elution

With **mdi** Viral RNA/DNA Miniprep Kit, highly concentrated RNA is obtained with 40µl of buffer RDE.

* 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 200µl.

Centrifugation:

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

Lysis

1. Check buffer RDL. In case of precipitation, re-dissolve by warming to 56°C for few minutes.
2. For efficient lysis, mix sample completely with buffer RDL, then incubate at 56°C for 20 minutes.

Washing

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

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/DNA, perform elution with 40µl of buffer RDE.

12.2 Protocol

Note: Things to do before starting

1. Prepare heating block or water bath at 56°C.

2. Arrange ethanol (96-100%).

3. Add recommended volume of **Ethanol (96-100%)** to buffer RDW1 and buffer RDW2 before use as shown in Table below.

Buffer	Buffer Volume	Volume of Ethanol(96-100%) to be added
RDW1	15.5ml	13.5ml
RDW2	14ml	36ml

4. Check buffer RDL. If precipitates are observed, incubate at 56°C in original bottle for few minutes until they redissolve. Cool buffer back to room temperature before use.

5. Dissolve carrier RNA completely in buffer RDE by adding 310µl of buffer RDE to 310µg 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.

6. **Addition of Carrier RNA to Buffer RDL:** For every test to be conducted, 5.6µl of carrier RNA stock solution of 1µg/µl from step 5 above needs to be added to 500µl of buffer RDL. **Buffer RDL-carrier RNA solution** should be prepared fresh, but for convenience, if the frequency of testing/use of kit reactions is high, the 310µl of carrier RNA stock solution from step 5 above may be fully added to bottle containing 31ml of buffer RDL. **Buffer RDL-carrier RNA solution** is stable for up to 48 hours if stored at 2-8°C and would be good for more than 50 tests.

   Note: If precipitation is observed after storing the **Buffer RDL-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.

7. Use RNase/DNase free plastics and glasswares.

Procedure:

1. Pipet 30µl Proteinase K into the bottom of a 1.5ml microcentrifuge tube.

2. Add 200µl sample to the microcentrifuge tube and mix well by pipetting up and down.

   Note: It is possible to add Proteinase K to the sample that have already been dispensed into microcentrifuge tube. The volumes of samples less than 200µl should be adjusted to 200µl with phosphate buffered saline (PBS) and low viral titre should be concentrated to 200µl before processing.

3. Add 500µl buffer RDL (containing 5.6µg Carrier RNA) to the sample, mix throughly by vortexing.

   Note: Do not add Proteinase K directly to buffer 'RDL'.

4. Incubate at 56°C for 20 minutes.

5. Briefly centrifuge the tube to remove drops from inside of the lid.

6. 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.

7. Place the spin column in the collection tube. Pass the above 700µl solution from step 6 through the spin column by spinning at $\geq$ 10,000rpm for 1 minute. Discard the flow through but save the collection tube.

8. Repeat step 7 with the remaining solution.

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

9. Place the spin column in the same collection tube. Wash the column with 500µl of buffer RDW1 by centrifuging for 1 minute at ≥ 10,000rpm. Discard the flow through but save the collection tube.

10. Place the spin column in the same collection tube. Wash the column with 500µl of buffer RDW2 by centrifuging for 1 minute at ≥ 10,000rpm. Discard the flow through but save the collection tube.

11. Place the spin column in the same collection tube. Wash the column with 400µl of buffer RDW2 by centrifuging for 2 minutes at ≥ 10,000rpm. Discard the flowthrough and collection tube.

12. Place the spin column in a RNase/DNase free 1.5ml microcentrifuge tube (not provided). Elute the bound viral RNA/DNA by adding 40µl buffer RDE directly to the center of the column membrane and let it stand for **5 minutes**. Spin at ≥ 10,000rpm for 1 minute.

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

    Note: Store RNA/DNA at -20°C to -80°C.

13. Trouble Shooting Guide

A. Little or no Yield of Viral RNA/DNA

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 56°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 RDL and ethanol before loading on to the spin column	Add buffer RDL 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/DNA

1. Nuclease contamination	Use RNase/DNase free plastics and glasswares.
2. Sheared RNA/DNA	Avoid vigorous mixing.

C: RNA/DNA does not perform well

1. Residual wash buffer in eluate	After second washing with RDW2, discard the flowthrough and place the spin column in the same collection tube with closed lid. Centrifuge for additional 2-3 minutes at ≥10,000rpm.

2.	Centrifugation less than 10,000rpm in drying step.	Centrifuge at ≥10,000rpm in drying step. If centrifuge rpm is less than 10,000rpm then discard the flowthrough after second washing with RDW2, 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
VRDK	VRDK					100	0100

Example:

VRDK	XX	XX	XX	X	0100

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UGL VRDK502000L