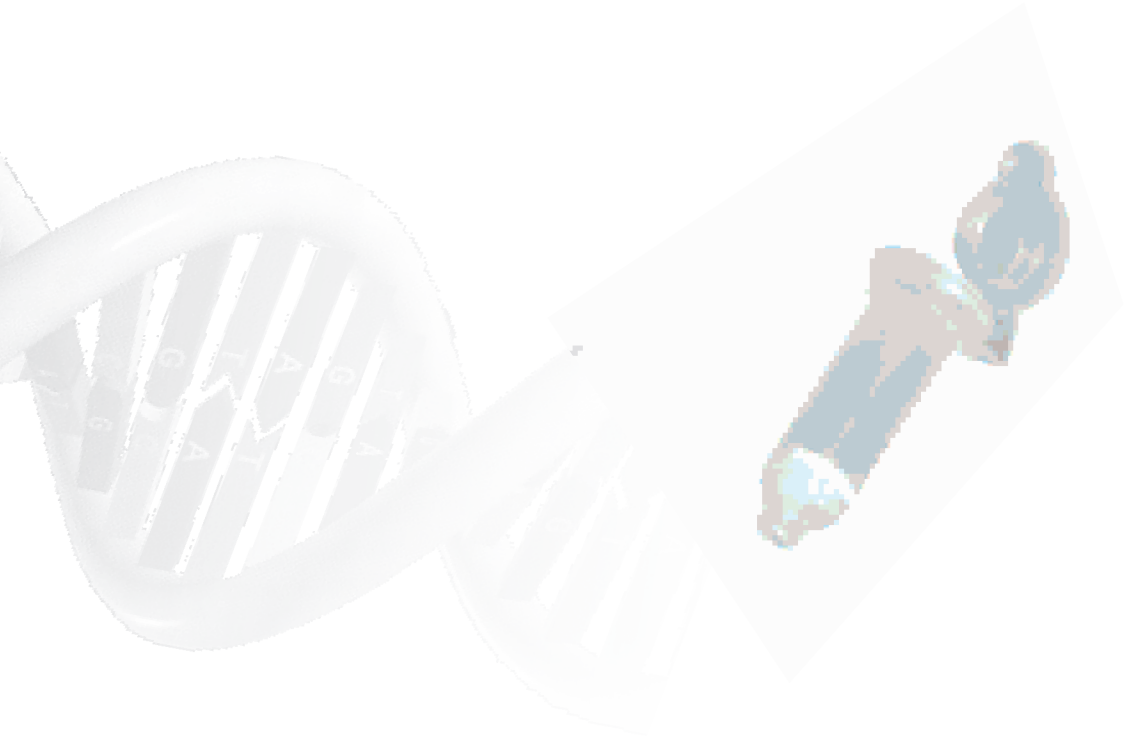
Viral RNA/DNA Miniprep Kit From Plasma



User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Viral RNA/DNA from Plasma miniprep Kit is a fast, economical and easy isolation method of high purity Viral RNA/DNA from plasma. The buffer system provided in the kit allows efficient lysis followed by selective binding of Viral RNA/DNA to the spin column.

Purified Viral RNA/DNA is eluted in low-salt buffer for variety of downstream applications. This technology does away with the cumbersome methodologies of phenol chloroform extraction as well as alcohol precipitation.

2. Applications

Purified Viral RNA/DNA 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 56°C for sometime and cool it down to room temperature.

4. Quality Assurance

The **mdi** Viral RNA/DNA from Plasma 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 certified by Underwriters Laboratories, USA for ISO 9001: 2008 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 from Plasma miniprep Kit

6. Lot Release Criteria

Each lot of Viral RNA/DNA from Plasma 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

* Stimulate 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.

8. Kit Contents

Contents	Quantity				Storage Temperature
Spin Columns	25	50	250	1000	RT
Collection Tubes	25	50	250	1000	RT
Buffer RDL	7.5ml	15ml	75ml	300ml	RT
Buffer RDW1	7.75ml	15.5ml	77.5ml	310ml	RT
Buffer RDW2	7ml	14ml	70ml	280ml	RT
Buffer RDE	10ml	20ml	100ml	400ml	RT
Proteinase K	16mg	32mg	160mg	640mg	2-8°C
Proteinase K buffer	800µl	1.6ml	8ml	32ml	2-8°C
Hand Book	1	1	1	1	–
Certificate of Quality	1	1	1	1	–

Note:

1. Reconstitute Proteinase K in proteinase K buffer (provided) to prepare stock of 20mg/ml
2. **Add recommended volume of Ethanol (96-100%)to buffer RDW1 and buffer RDW2 before use(See page 10).**

9. Specifications

Maximum Volume of Plasma Sample	200µl
Capacity of column reservoir	700µl
Binding capacity of membrane (RNA/DNA)	10µg
Elution volume	40 µl

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

1. Do not subject the stored samples to repeated freezing and thawing as it leads to reduced RNA/DNA size
2. Poor-quality starting material leads to reduced length and yield of purified RNA/DNA.

B. Maximum Amount of Starting Material

Do's

Maximum starting sample should be 200µl.

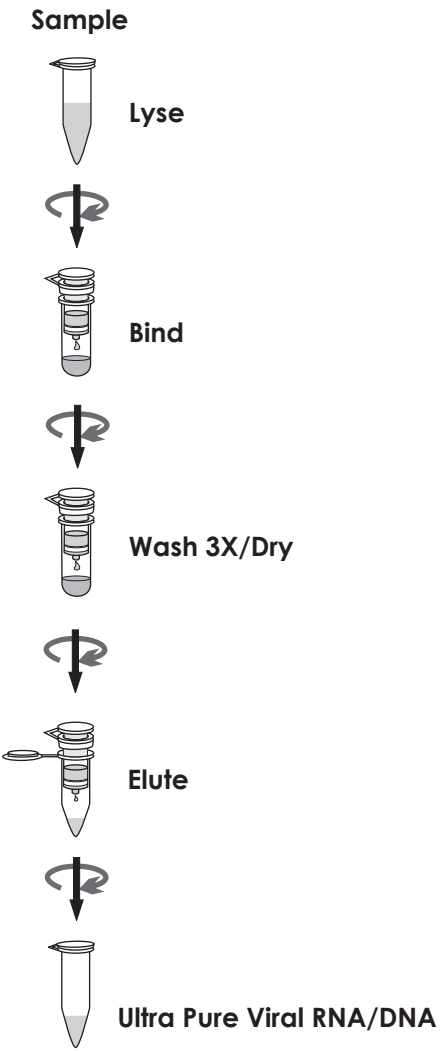
Don'ts

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

C. Elution

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

11. mdi Viral RNA/DNA from Plasma miniprep Procedure



12. Viral RNA/DNA from Plasma Miniprep

12.1 Principle

Obtaining highly pure Viral RNA/DNA from plasma using **mdi** Viral RNA/DNA from Plasma miniprep Kit involves:

Lysis of Plasma Sample

For efficient lysis, plasma 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 Viral RNA/DNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away.

Washing is performed with buffer 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 40 µl of buffer RDE.

12.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of Plasma sample should be 200µl.

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 plasma sample completely with Proteinase K and buffer RDL, then incubate at 56°C.
2. Proteinase K should be added to sample before addition of buffer RDL to inactivate nuclease that can degrade RNA/DNA during purification.
3. In case of any precipitation in buffer RDL, re-dissolve by warming to 56°C for few minutes.
4. Ensure that recommended Volume of Ethanol(96-100%) has been added to buffer RDW1 and RDW2.

Washing

1. To remove residual wash buffer, spin the column with closed lid for 2 minutes 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 Viral RNA/DNA, perform elution with 40 µl of buffer RDE.

12.3 Protocol

Note: Things to do before starting

1. Heat a water bath at 56°C
2. Arrange ethanol (96-100%)
3. Use all plastic wares RNase A/ Dnase A free.
4. Add recommended volume of ethanol (96-100%) in buffer RDW1 and RDW2 then mix well.
5. Reconstitute proteinase K in proteinase K buffer (provided) to prepare stock of 20mg/ml

Spin column	Buffer	Buffer Volume	Volume of Ethanol(96-100%) to be added
25	RDW1	7.75ml	6.75ml
50	RDW1	15.5ml	13.5ml
250	RDW1	77.5ml	67.5ml
1000	RDW1	310ml	270ml
25	RDW2	7ml	18ml
50	RDW2	14ml	36ml
250	RDW2	70ml	180ml
1000	RDW2	280ml	720ml

Procedure:

1. Pipet 30µl Proteinase K into the bottom of a 1.5ml microcentrifuge tube.
2. Add 200µl plasma 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.
3. Add 200µl buffer RDL to the sample, mix throughly by vortexing.

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

4. Incubate at 56°C in water bath for 20 minutes. Mix 2-3 times by inverting the tube during incubation.
5. Briefly centrifuge the sample after incubation to remove drops from inside of lid.
6. Add 250µl ethanol(96-100%), mix well by vortexing and briefly centrifuge the sample to remove drops from inside of lid.
7. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥ 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 balance and spin at ≥ 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.
8. 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 flowthrough.
9. 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 flowthrough.
10. Place the spin column in the same collection tube. Wash the column with 400µl of buffer RDW2 by centrifuging for 2 minute at ≥ 10,000rpm. Discard the flowthrough, and collection tube.
11. Place the spin column in a RNase A/DNase A free 1.5ml microcentrifuge tube (not provided)
12. Elute the bound Viral RNA/DNA by adding 40 µl of buffer RDE directly to the center of the column membrane and let it stand for 5 minutes at room temperature . Spin at ≥ 10,000rpm for 1 minute.

    Note: RNA as well as RNA/DNA should be stored at -80°C.

13. Trouble Shooting Guide

A. Little or no Yield of Viral RNA/DNA

1. Overloading of spin column	Can happen if the starting sample is more than the recommended amount.
2. Precipitates in buffer	In case of any precipitates in buffers, re- dissolve by warming to 56°C before use.
3. Spin column choked	Increase centrifugation time while passing sample through the spin column.
4. Insufficient Lysis	Check that Proteinase K was added to the sample prior to addition of buffer RDL. Ensure incubation temperature of 56 °C after addition of buffer RDL.
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 another 5 minutes.
7. Insufficient mixing of sample with buffer RDL and ethanol before loading on to the spin column	Do additional vortexing for 15 seconds
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 plasma samples	For short term storage plasma samples should be stored at 4°C in tubes containing a standard anticoagulant (E.D.T.A.). For long term, store at-70°C with anticoagulant.

B: Low quality RNA/DNA

1. Nuclease contamination	Use Rnase A/ DNase A free plastic and glassware.
2. Sheared Viral RNA/DNA	Avoid vigorous mixing.

C: RNA/DNA does not perform well

Residual wash buffer in eluate	After second wash with buffer RDW2, 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.

D: Low A_{260}/A_{280} ratio

RNA/DNA diluted with water instead of buffer	Use buffer RDE to dilute the sample before measuring A_{260}/A_{280} ratio for purity.

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 30 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	Pack Size

Type	Code
VRDK	VRDK

Pack Size	Code
50	0050
250	0250
1000	1000

Example:

VRDK	XX	XX	XX	X	0250