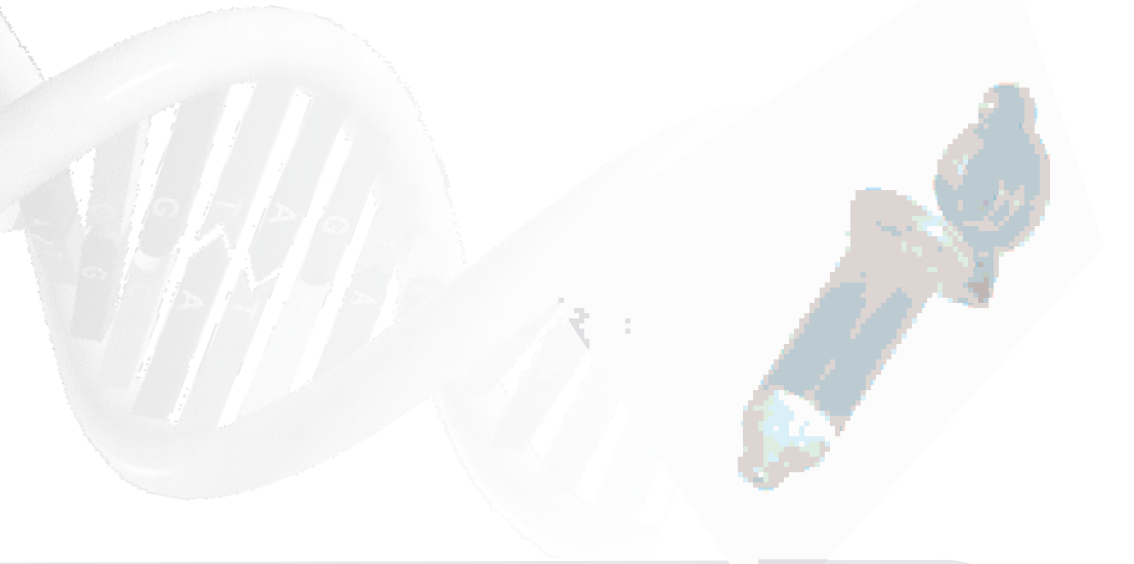
Viral DNA From Plasma Miniprep Kit



User Guide

mdi
Membrane Technologies

ADVANCED MICRODEVICES PVT. LTD.
21, Industrial Area, Ambala Cantt - 133006 (INDIA)
Tel: 0171-2699290, 2699471, 2699274
Fax: +91-171-2699221, 2699008
Email: info@mdimembrane.com
Website: www.mdimembrane.com

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1. Introduction

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

Purified Viral 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 DNA is suitable for

1. 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 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 DNA from Plasma miniprep Kit

6. Lot Release Criteria

Each lot of Viral 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 DL	17.5ml	35ml	175ml	650ml	RT
Buffer DW1	7.75ml	15.5ml	77.5ml	310ml	RT
Buffer DW2	7ml	14ml	70ml	280ml	RT
Buffer DE	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 DW1 and buffer DW2 before use(See page 10).**

9. Specifications

Maximum Volume of Plasma Sample	200µl
Capacity of column reservoir	700µl
Binding capacity of membrane (ds 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 DNA size
2. Poor-quality starting material leads to reduced length and yield of purified DNA.

B. Maximum Amount of Starting Material

Do's

Maximum starting sample should be 200µl.

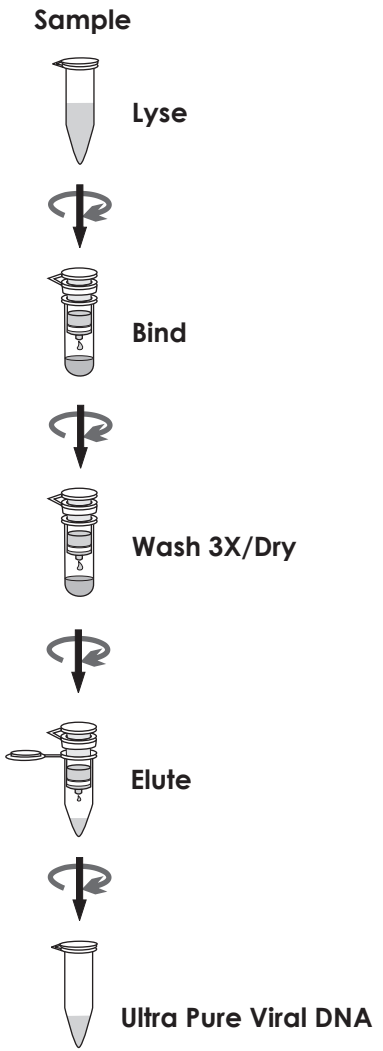
Don'ts

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

C. Elution

With **mdi** Viral DNA from Plasma miniprep Kit, highly concentrated Viral DNA is obtained with 40 µl of pre-heated (at 70 - 75°C for 30 minutes in an oven)elution buffer DE.

11. mdi Viral DNA from Plasma miniprep Procedure



12. Viral DNA from Plasma Miniprep

12.1 Principle

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

Lysis of Plasma Sample

For efficient lysis, plasma sample is mixed with Proteinase K and buffer DL, and incubated at 56°C.

Capturing of Viral DNA on Spin Column

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

Washing

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

Washing is performed with buffer DW1 and DW2.

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 'DE', pre-heated at 70-75°C for 30 minutes in an oven. For obtaining highly concentrated Viral DNA, perform elution with 40 µl of buffer DE.

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 DL, then incubate at 56°C.
2. Proteinase K should be added to sample before addition of buffer DL to inactivate nuclease that can degrade DNA during purification.
3. In case of any precipitation in buffer DL, re-dissolve by warming to 56°C for few minutes.
4. Ensure that recommended Volume of Ethanol(96-100%) has been added to buffer DW1 and DW2.

Washing

1. To remove residual wash buffer, spin the column with closed lid for 2 minutes at ≥ 10,000 rpm.

Elution

1. Use pre-heated (at 70 - 75°C for 30 minutes in an oven) buffer DE.
2. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 5-6 minutes.
3. For obtaining highly concentrated Viral DNA, perform elution with 40 µl of buffer DE.

12.3 Protocol

Note: Things to do before starting

1. Heat a water bath at 56°C
2. Arrange ethanol (96-100%)
3. Preheat the elution buffer DE at 70-75°C for 30 minutes in an oven
4. Add recommended volume of ethanol (96-100%) in buffer DW1 and DW2 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	DW1	7.75ml	6.75ml
50	DW1	15.5ml	13.5ml
250	DW1	77.5ml	67.5ml
1000	DW1	310ml	270ml
25	DW2	7ml	18ml
50	DW2	14ml	36ml
250	DW2	70ml	180ml
1000	DW2	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 DL to the sample, mix throughly by vortexing.

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

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 230µl ethanol, 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 DW1 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 DW2 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 DW2 by centrifuging for 2 minute at ≥ 10,000rpm. Discard the flowthrough, and collection tube.
11. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)
12. Elute the bound Viral DNA by adding 40 µl of pre-heated(70-75°C) buffer DE directly to the center of the column membrane and let it stand for 5 minutes. Spin at ≥ 10,000rpm for 1 minute.

13. Trouble Shooting Guide

A. Little or no Yield of Viral 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 DL. Ensure incubation temperature of 56 °C after addition of buffer DL.
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 DL 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 DNA

1. Nuclease contamination	Use autoclaved plastic and glassware.
2. Sheared Viral DNA	Avoid vigorous mixing.

C: DNA does not perform well

Residual wash buffer in eluate	After second wash with buffer DW2, 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

DNA diluted with water instead of buffer	Use buffer DE 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**					**Pack Size**	**Code**
VDPK	VDPK					50	0050
						250	0250
						1000	1000

Example:

VDPK	XX	XX	XX	X	0250

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