Medi-R Total RNA PLUS Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Medi-R Total RNA Plus Miniprep Kit is designed to have a fast, easy and economical isolation of upto 10µg of high purity total RNA from buffy coat or leukocyte pellet. The **mdi** Medi-R Total RNA Plus Miniprep Kits are targeted to purify RNA from small amounts of starting material. A maximum amount of 1 X 10^7 leukocytes can be generally processed. The kit incorporates a uniquely formulated buffer M-Zole to lyse leukocytes and fast spin column technology to purify it in less than 30 minutes. **mdi** Medi-R Total RNA Plus Miniprep Kit integrates phenol-chloroform based sample preparation.

2. Downstream Applications

1. RT-PCR and Real Time RT-PCR
2. Differential Display
3. cDNA Synthesis
4. Northern, Dot, and Slot Blot Analysis

3. Storage Conditions

mdi Medi-R Total RNA Plus Miniprep Kit should be stored dry at room temperature (15 - 25 °C). The kit is stable for one year at above storage conditions without showing any reduction in performance and quality.

For longer storage, the entire kit can be stored at 2-8°C. In case precipitates are observed in buffer, re-dissolve all buffers before use at 37°C for few minutes. All buffers should be at room temperature before starting the protocol.

4. Quality Assurance

The **mdi** Medi-R Total RNA Plus 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 **mdi** Medi-R Total RNA Plus Miniprep Kit.

6. Lot Release Criteria

Each lot of **mdi** Medi-R Total RNA Plus 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 offer 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 system especially to satisfy your needs.

We welcome your feedback to improve our products.

8. Kit Contents

Contents	Quantity			Storage Temperature
Spin Columns	50	250	1000	RT
mdi RNA Shredder Spin Columns	50	250	1000	RT
Buffer RL	400ml	2000ml	8000ml	RT
Buffer M-Zole	55ml	260ml	1050ml	RT
Buffer RW1	30ml	150ml	600ml	RT
Buffer RW2	60ml	300ml	1200ml	RT
Buffer RE	10ml	50ml	200ml	RT
Collection Tubes	100	500	2000	RT
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

9. Specifications

RNA Binding Capacity	Up to 10µg
Capacity of column reservoir	750µl
Recovery	80%
Minimum elution volume	50µl
Total time taken	< 30 Minutes

10. Volumes for a Miniprep

Maximum Leukocyte Count	1 X 10^7
Buffer RL	7.5 ml
Buffer M-Zole	1ml
Buffer RW1	500µl
Buffer RW2	500µl x 2
Buffer RE	50µl

11. Principle

mdi Medi-R Total RNA Plus Miniprep Kit allows the isolation of ultra pure total RNA which involves:

1. Lysis
2. Homogenization
3. Preparation of Aqueous Layer
4. Capturing RNA on spin column
5. Washing
6. Elution

1. Lysis

During **mdi** Medi-R Total RNA Plus isolation procedure, leukocytes are efficiently lysed under selective conditions, thus inactivating RNases and allowing isolation of intact RNA. **mdi** RNA Shredder Spin Column allows fast and simple homogenization of cell lysates without the risk of cross-contamination.

2. Homogenization

Total cellular RNA isolation from buffy coat or from leukocyte pellet requires efficient disruption of cells and homogenization of lysate for optimal yield and purity. **mdi** Medi R Total RNA Plus Kit provide **mdi** shredder spin columns, which allows fast and simple homogenization of cell lysates. Cell lysates are loaded onto the **mdi** shredder spin column, centrifuged briefly and the homogenized lysate is collected in a 2ml collection tube.

3. Preparation of Aqueous Layer

After addition of chloroform, by centrifugation lysate is separated into

Colorless upper aqueous phase containing RNA, white interphase containing DNA, and lower red organic phase containing proteins etc.

The upper aqueous phase is collected and further processed. Then RNA is purified using **mdi** spin columns.

4. Capturing Total RNA on Spin Column

In order to facilitate adsorption of RNA onto the spin columns, suitable membrane is selected for the spin column along with buffer M-Zole and ethanol which binds the RNA onto it.

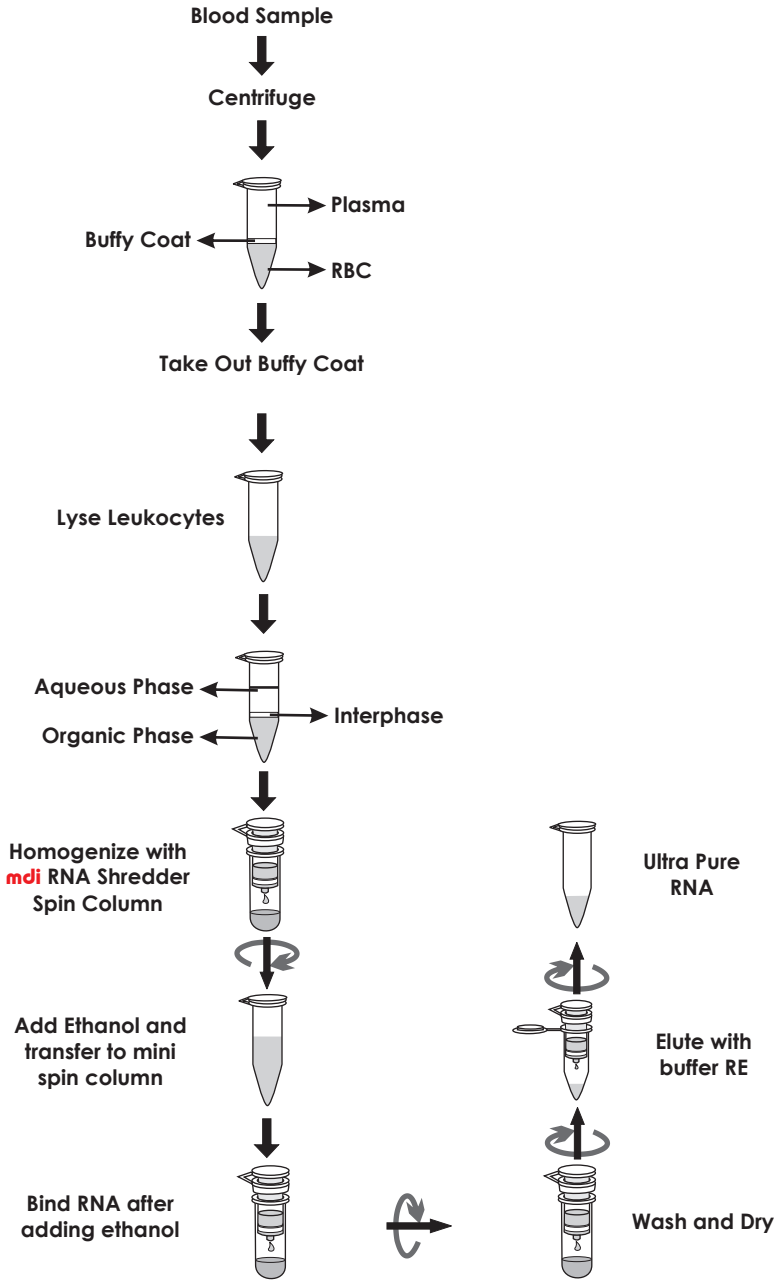
5. Washing

Subsequent to RNA binding onto the spin column, unwanted components like DNA, proteins and polysaccharides are washed away. Washing is done by buffer 'RW1' and 'RW2'. Unwanted components are not retained on membrane and passes in flowthrough.

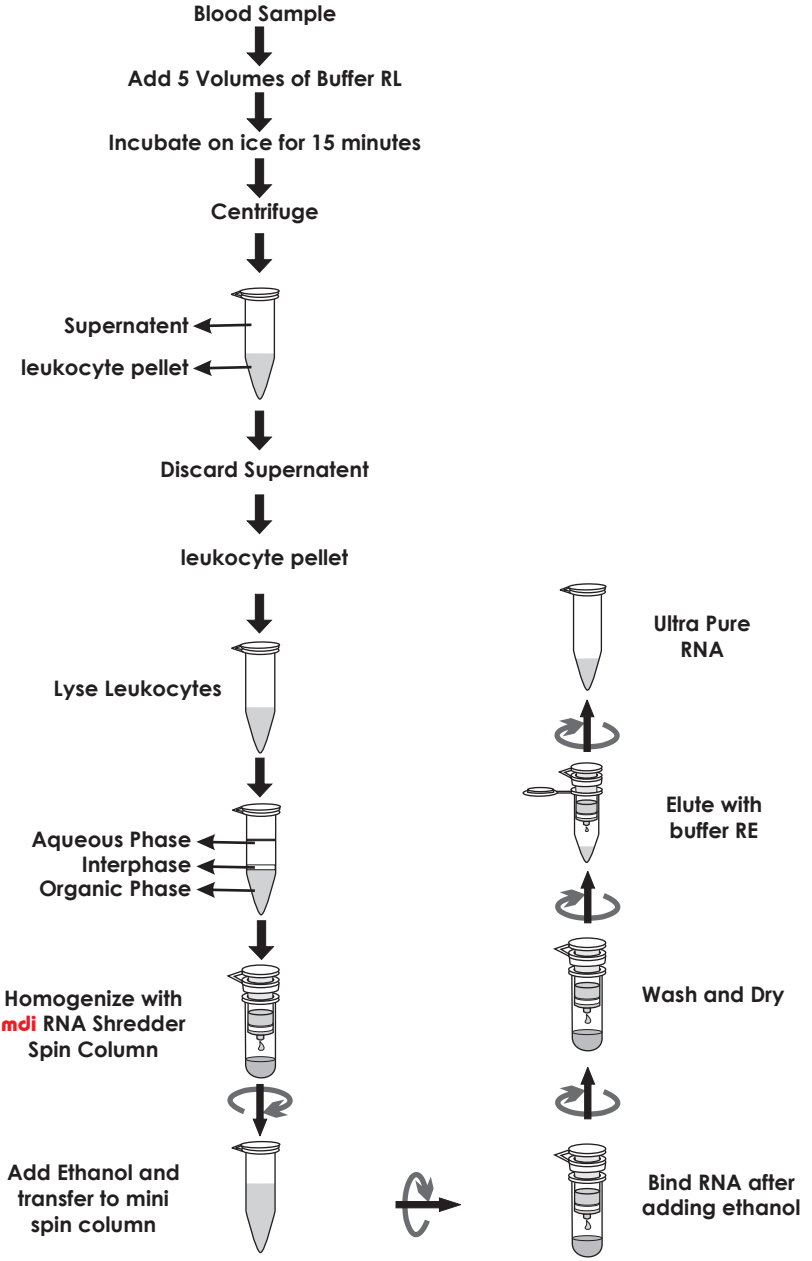
6. Elution

Salt concentration and pH of elution buffer is important for elution efficiency, elution occurs at basic conditions and low salt concentration. Elution is done with buffer 'RE'.

12. mdi Medi-R Total RNA Plus Miniprep Procedure : Option 1



13. mdi Medi-R Total RNA Plus Miniprep Procedure : Option 2



14. Protocol

14.1 Important Points Before Starting

1. It is essential to use correct amount of starting material in order to obtain optimal RNA yield and purity. A maximum amount of 1 X 10^7 leukocytes can be generally processed.
2. Do not overload the column as this will significantly reduce the RNA yield and quality.
3. **Homogenization** is necessary to reduce the viscosity of the lysates. Incomplete homogenization results in inefficient binding of RNA to the **mdi** spin column membrane and therefore significantly reduced RNA yields.
4. Arrange (96-100%) ethanol and chloroform.
5. Arrange 1.5ml or 2 ml RNase free microcentrifuge tubes.
6. All plastic wares and glass wares should be RNase - free.
7. Buffers may precipitate upon storage. Redissolve by warming at 37°C, and then bring it to room temperature (15 - 25°C) for use.

14.2 Procedure

1. Prepare buffy coat containing not more than than 1 X 10^7 cells.

   Note : In case the blood sample has very low leukocyte count it is recommended to follow the following steps (a,b,c) instead of preparing buffy coat. Then continue from step 2.

a) Add 5 volumes of Buffer RL to 1 volume of human whole blood (up to 1.5ml) in an appropriately sized centrifuge tube (not provided). Mix well.

b) Incubate for 15 minutes on ice. Mix by inverting 2-3 times during incubation. The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes

c). Centrifuge at 3000rpm for 10 minutes and discard the supernatant carefully by inverting the tube. Leukocytes will form a pellet after centrifugation. Ensure supernatant is completely removed.

2. Add 1ml of buffer M-Zole and mix buffy coat or leukocyte pellet by pipetting up and down or by vortexing.

3. Incubate at room temperature for 5 minutes.

4. Transfer the lysate (approx. 700µl) to **mdi** RNA shredder spin column placed in a 2 ml collection tube and centrifuge at ≥ 10,000rpm for 1 minute. Then transfer the flow through carefully from the collection tube in to the new RNase free 1.5ml microcentrifuge tube (not provided).

5. Place the above **mdi** RNA shredder in the same collection tube and load the remaining lysate. Centrifuge at ≥ 10,000 rpm for 1 minute. Then transfer the flow through to the same RNase free 1.5ml centrifuge tube.

6. Add 200µl chloroform. Close the cap and mix it vigorously for 30 seconds.

7. Incubate the lysate at room temperature for 2-3 minutes.

8. Centrifuge at 10,000rpm for 15 minutes at room temperature.

   *The sample separates into three phases after centrifugation an upper colorless aqueous phase contains RNA, a white interphase, and a lower red organic phase.

9. Carefully transfer the upper aqueous phase approx. 600µl to a new RNase free microcentrifuge tube (not supplied).

   Leave 50-100µl of aqueous phase to eliminate DNA contamination.

10. Add 1 volume (approx. 600µl) of (96-100%)ethanol and mix throughly by pipetting up and down.

11. Transfer the lysate, including any precipitate that may have formed, to **mdi** Mini Spin Column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 15 seconds at ≥10,000rpm. Discard the flowthrough. Reuse the collection tube.

    Note: Maximum volume of column reservoir is 750µl. For sample volumes >750µl, simply load the remaining sample, balance the microcentrifuge and spin again. Discard the flowthrough.

12. Place the spin column in the same collection tube. Wash the spin column with 500µl of buffer RW1 by centrifuging for 15 seconds at ≥10,000rpm. Discard the flowthrough.

13. Place the spin column in the same collection tube. Wash the spin column with 500µl of buffer RW2 by centrifuging for 15 seconds at ≥10,000rpm. Discard the flowthrough.

14. Place the spin column in the same collection tube. Wash the spin column with 500µl of buffer RW2 by centrifuging for 1 minute at ≥10,000 rpm.

15. Discard the flowthrough and place the spin column with closed lid in the same collection tube, centrifuge at ≥10,000rpm for 2 minutes. Discard the collection tube.

16. Place the spin column in a fresh RNase free 1.5ml microcentrifuge tube (not provided). Add 50µl of buffer RE or RNase free water directly to the center of the spin column membrane. Close the lid gently, incubate at room temperature for 1 minute, and then centrifuge for 1 minute at ≥10,000 rpm.

15. Trouble Shooting Guide

A. Incomplete Erythrolysis with Buffer RL

1. The cloudy suspension does not become translucent.	Increase incubation time on ice to 20 minutes

B. mdi Mini Spin Column choked

1. Use of excess starting material	Repeat the procedure with the correct amount of starting material.
2. Lysate was not processed with **mdi** Shredder Spin Column	Pass the lysate after adding and mixing of Buffer M-Zole through the **mdi** RNA Shredder Spin Column.

C. Low RNA Yield

1. Use of excess starting material	The starting material should not be more than recommended.
2. Spin column choked	The column can choke in case the lysate is not clear before loading. Use the lysate after processing with **mdi** RNA Shredder Spin Column
3. Improper dispensing of elution buffer	The elution buffer must be dispensed properly on to the center of the column membrane.
4. Insufficient incubation of elution buffer in the column membrane.	Increase incubation time by 2 - 3 minutes.

D. Low A_{260}/A_{280} value

1. Water used to dilute RNA for A_{260}/A_{280}	Use Buffer RE to dilute the sample before measuring A_{260}/A_{280} ratio for purity.

E. Low quality RNA

1. Degraded RNA	Use RNase free plastic and glasswares. Use fresh blood for preparation of buffy coat or leukocyte pellet.
2. Residual DNA	DNA contamination can happen if the interphase has been disturbed. Leave 50-100µl of aqueous phase.For complete removal of DNA, Digest the eluate with DNase & inactivation by heat before use in downstream

F. RNA does not perform well

1. Residual wash buffer in eluate	After discarding flowthrough, spin the column with closed lid for 1-2 minutes extra at $\geq$10,000 rpm.

16. 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.

17. 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 quality 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.

17. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
TPRK	TPRK					50	0050
						250	0250
						1000	1000

Example:

TPRK	XX	XX	XX	X	0250

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