Mammalian Tissue Total RNA Miniprep Kit

User Guide

mdi
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1. Introduction

mdi Mammalian Tissue Total RNA Miniprep Kit is designed to have a fast, easy and economical isolation of upto 100µg of high purity total RNA from tissues. The **mdi** Mammalian Tissue Total RNA Miniprep Kits are targeted to purify RNA from small amounts of starting material. A maximum amount of 25-30mg tissue can be generally processed. The kit incorporates a uniquely formulated buffer RG to lyse tissue and fast spin column technology to purify it in less than 30 minutes. This technology does away with phenol extraction (associated with desalting) and ethanol precipitation (associated with anion exchange based purification).

2. Downstream Applications

1. RT-PCR and Real Time RT-PCR
2. Differential Display
3. cDNA Synthesis
4. Northern, Dot, and Slot Blot Analysis
5. Primer Extension
6. Micro Array

3. Storage Conditions

mdi Mammalian Tissue Total RNA 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** Mammalian Tissue Total RNA 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** Mammalian Tissue Total RNA Miniprep Kit.

6. Lot Release Criteria

Each lot of **mdi** Mammalian Tissue Total RNA 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
Collection Tubes	100	500	2000	RT
RNA Preserver	50	250	1000	RT
Buffer RG	35ml	175ml	700ml	RT
Buffer RW1	30ml	150ml	600ml	RT
Buffer RW2	60ml	300ml	1200ml	RT
Buffer RE	30ml	150ml	600ml	RT
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

Note: For On-Column DNase digestion, DNase is not provided in the kit. It can be purchased from **mdi** separately.

9. Specifications

RNA Binding Capacity	Up to 100µg
Capacity of column reservoir	750µl
Recovery	80%
Minimum elution volume	50µl
Total time taken	< 30 Minutes

10. Volumes for a Miniprep

Maximum Tissue Sample	25-30mg
Buffer RG	600µl
Buffer RW1	500µl
Buffer RW2	500µl x 2
Buffer RE	50µl

11. Principle

mdi Mammalian Tissue Total RNA Miniprep Kit allows the isolation of ultra pure total RNA which involves:

1. Lysis
2. Capturing RNA on spin column
3. Washing
4. Elution

1. Lysis

During mdi Mammalian Tissue Total RNA isolation procedure, Tissue is ground under liquid nitrogen to a fine powder using a pre cooled mortar and pestle . Ground tissue is efficiently lysed under selective condition, Thus inactivating RNases and allowing isolation of intact RNA. mdi RNA shredder spin column allows fast and simple homogenization of lysate without the risk of cross contamination.

2. 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 RG and ethanol which binds the RNA onto it.

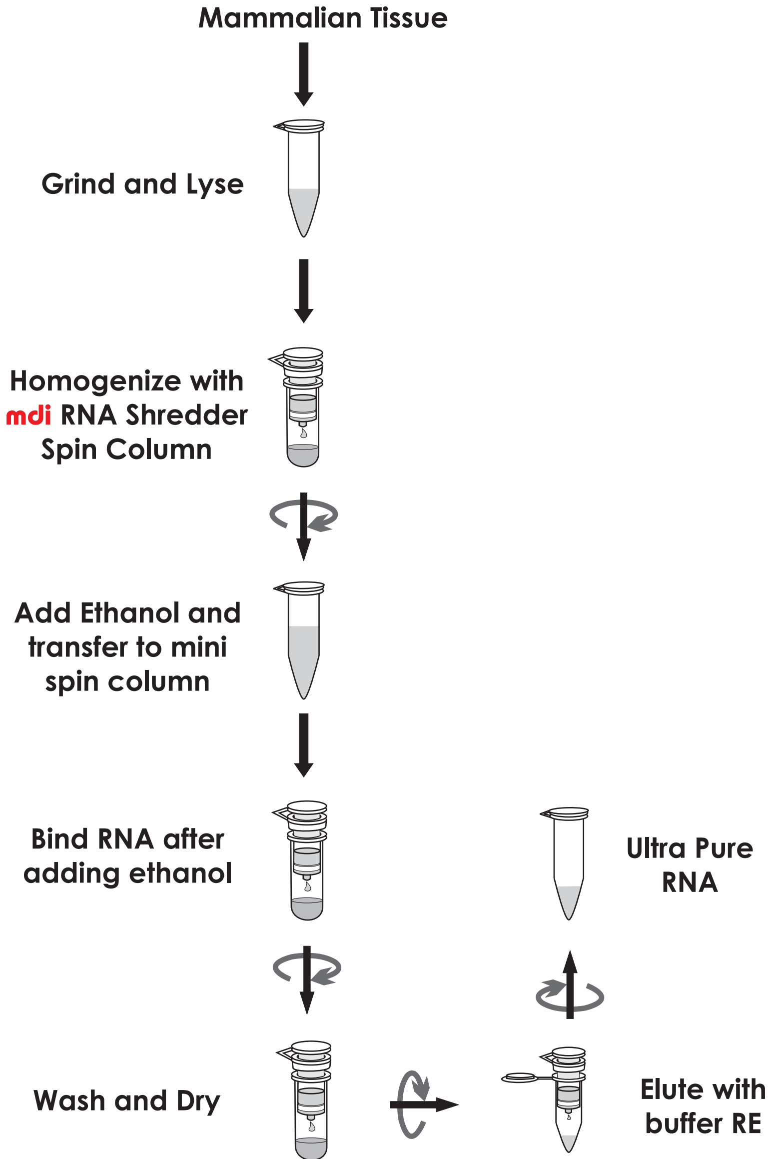
3. Washing

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.

4. 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 Mammalian Tissue Total RNA Miniprep Procedure



13. Protocol

13.1 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 Tissue (25-30mg) can be generally processed.

   Optional: Freshly dissected tissue can be preserved in RNA preserver. For this cut the tissue into small pieces of thickness less than 0.5mm, submerge the tissue in RNA preserver and store at 2-8°C.

   For storage at -20°C incubate the tissue overnight in the RNA preserver at 2-8 °C. Then transfer to -20°C for storage.

   For storage at -80°C incubate the tissue overnight in the RNA preserver at 2-8 °C. remove the tissue from RNA preserver and transfer to -80° for storage

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 commercially available β–mercaptoethanol.

5. Prepare 70% ethanol for step-6.

6. Add 10 µl of β-mercaptoethanol per 1ml buffer RG and mix well. Buffer RG is stable at room temperature for 1 month after addition of β-mercaptoethanol.

7. All plastic wares and glass wares should be RNase - free.

8. Buffers may precipitate upon storage. Redissolve by warming at 37°C, and then bring it to room temperature (15 - 25°C) for use.

13.2 Procedure

1. Excise the tissue sample from animal or remove it from storage. Remove RNA preserver stored tissue from the reagent with RNase free forceps
2. Weight 25-30mg tissue.
3. Grind the tissue under liquid nitrogen to a fine powder using a precooled mortar and pestle. transfer the powder and liquid nitrogen into a RNase free microcentrifuge tube and allow the liquid nitrogen to evaporate.
4. Add buffer RG 600µl containing β -mercaptoethanol (10µl/ml) and resuspend completely by vortexing.
5. Transfer the lysate to **mdi** RNA shredder spin column placed in a 2 ml collection tube and centrifuge at ≥ 10,000rpm for 1 minute. Then transfer the supernatant carefully from the collection tube in to the new RNase free 1.5ml microcentrifuge tube without disturbing the pellet.
6. Add 1volume of 70% ethanol to the cleared lysate and mix immediately by pipetting up and down.
7. 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.

   Optional: If performing optional **On-Column DNase digestion** (see Page 11), follow steps R1 - R4 instead of this step 8.

8. 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.

9. 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.

10. 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.

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

11. Place the spin column in a 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.

13.3 Optional On-Column DNase Digestion

Prepare and load samples onto **mdi** spin column. Instead of performing the first wash step (Step 8, Page 9), follow steps R1 - R4 below:

R1. Add 250µl Buffer RW1 to the **mdi** spin column. Close the lid gently and centrifuge for 15 seconds at ≥10,000rpm to wash the spin column membrane. Discard the flowthrough.

R2. Preparation of DNase I Incubation Mix:

Add 5µl of DNase I Stock Solution and 5µl of 10X buffer to 40µl of RNase free water in a RNase free tube. Mix by gently inverting the tube. Centrifuge for 15 seconds at ≥ 10,000 rpm to collect residual liquid from sides of the tube.

Note: DNase I is sensitive to physical denaturation. Mixing should be carried out only by inverting the tube. Do not vortex.

R3. Add the DNase I incubation mix (50µl) directly to **mdi** spin column membrane and incubate for 15 minutes at room temperature.

Note: Add DNase I incubation mix directly to **mdi** spin column membrane carefully. DNase digestion will be incomplete if part of the mix sticks to the walls.

R4. Add 250µl Buffer RW1 to the **mdi** spin column. Close the lid gently and centrifuge for 15 seconds at ≥ 10,000rpm. Discard the flow through. Continue with step 9 in the protocol (see Page 10)

14. Trouble Shooting Guide

A. mdi Mini Spin Column choked

Problem	Solution
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 RG through the mdi RNA Shredder Spin Column and use only the supernatant from the collection tube without disturbing the pellet.

B. Low RNA Yield

Problem	Solution
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.

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

Problem	Solution
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.

D. Low quality RNA

Degraded RNA	Use RNase free plastic and glasswares. Use β-mercaptoethanol in Buffer RG
Residual DNA	Digest the eluate with DNase and inactivate by heat before use in downstream or perform on column DNase digestion

E. RNA does not perform well

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

15. 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.

16. 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**
MTRK	MTRK					50	0050
						250	0250
						1000	1000

Example:

MTRK	XX	XX	XX	X	0250

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