Bacterial Total RNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Bacterial Total RNA Miniprep Kit is designed to have a fast, easy and economical isolation of upto 100µg of high purity total RNA from bacterial cultures (both from Gram Positive and Gram Negative bacteria). The mdi Bacterial Total RNA Miniprep Kits are targeted to purify RNA from small amounts of starting material. The kit incorporates a uniquely formulated buffer RG to lyse the bacterial culture 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 Bacterial 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 Bacterial 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 Bacterial Total RNA Miniprep Kit.

6. Lot Release Criteria

Each lot of mdi Bacterial 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
Buffer TE	30ml	150ml	600ml	RT
Buffer RG	45ml	250ml	1000ml	RT
Buffer RW1	45ml	250ml	1000ml	RT
Buffer RW2	60ml	300ml	1200ml	RT
Buffer RE	20ml	100ml	400ml	RT
Collection Tubes	50	250	1000	RT
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

9. Specifications

RNA Binding Capacity	≥100µg
Capacity of column reservoir	700µl
Recovery	80%
Minimum elution volume	50µl
Total time taken	< 30 Minutes

10. Volumes for a Miniprep

Number of Bacterial Cells	$5.8x10^8$ - $7.5x10^8$ Cells
Buffer RG	700µl
Buffer RW1	700µl
Buffer RW2	500µl x 2
Buffer RE	50µl

11. Principle

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

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

1. Lysis of Bacterial Culture

To efficiently lyse the bacterial culture, centrifuge it properly before addition of buffer TE. The lysed culture is then neutralized with the help of buffer RG.

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 washed away and pass into the flowthrough.

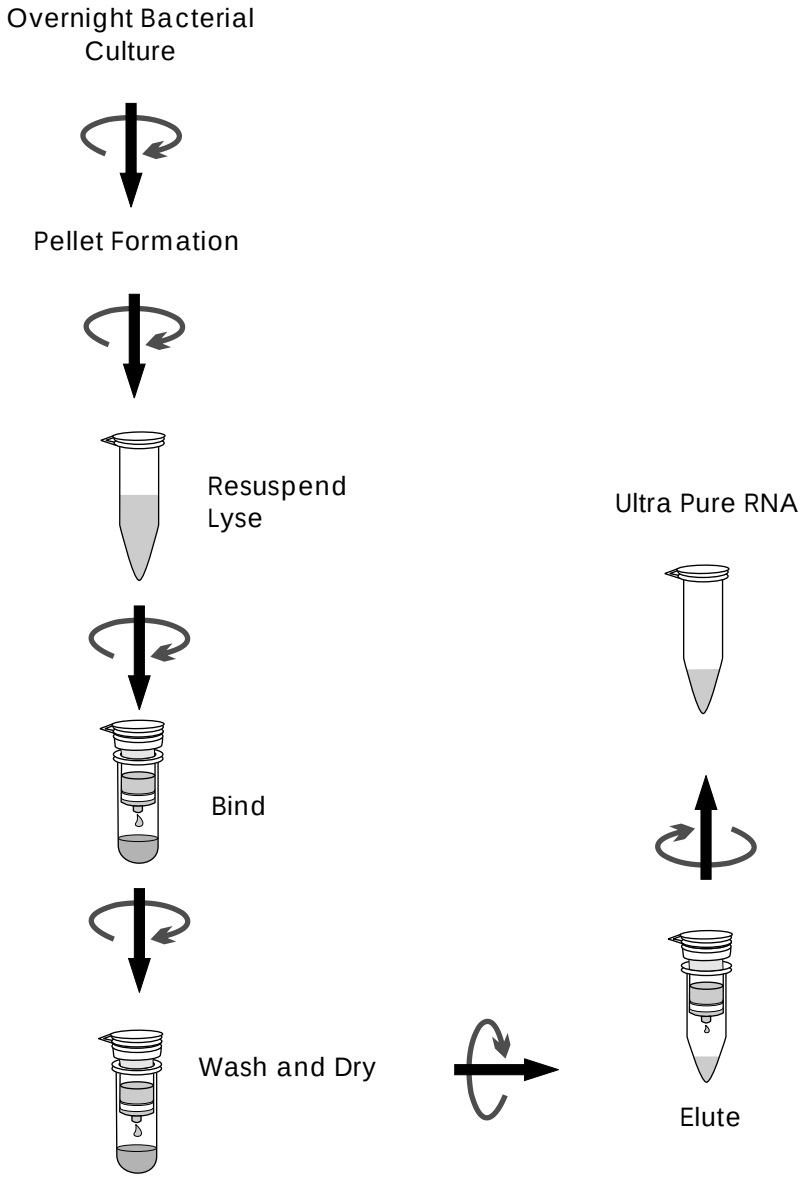
4. Elution

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'.

5. Yield (Typical Data)

Bacterial Strain	Medium	No. of Cells	RNA Yield (µg)
E. coli (DH_5a)	LB	$5x10^8$	53.5
Bacillus subtilis	LB	$1x10^8$	14.1
Bacillus subtilis	LB	$2x10^8$	26.2

12. mdi Bacterial Total RNA Miniprep Procedure



13. Protocol (Total RNA purification from Gram Negative Bacteria)

13.1 Important Points Before Starting

1. Prepare water bath or oven at 37 °C
2. Arrange (96-100%) ethanol and commercially available b-mercaptoethanol.
3. Arrange cell wall lysing enzyme eg. lysozyme etc.
4. All plastic wares and glass wares should be RNase free.
5. Bacterial lysis in this protocol consists of treatment with 20mg/ml lysozyme for 15 min incubation at 37 °C which is optimal with E. coli.

   Enzymatic lysis is affected by bacterial species, cell number, and culture medium, it may be therefore necessary to adjust enzyme concentration, enzyme incubation time, or to treat with a different enzyme.
6. Add 10 µl of b-mercaptoethanol per 1ml buffer RG and mix well. Buffer RG is stable at room temperature for 1 month after addition of b-mercaptoethanol.

13.2 Procedure

1. Centrifuge 1-3 ml ($5x10^8$ - $7.5x10^8$) cells from an overnight bacterial culture at $\geq$10,000 rpm for 5 minutes. Remove all traces of supernatant by inverting the open tube until all medium has been drained.
2. Add 200µl of buffer TE containing lysozyme (20mg/ml)
3. Resuspend the pellet completely by pipetting up and down. No cell clumps should be visible after resuspension.
4. Incubate at 37 °C for 15 minutes. Mix by inverting the tube for 2-3 times during incubation. Incubation time can be extended without affecting the procedure, and may increase the RNA yield.

5 Add 700 µl of buffer RG containing b-mercaptoethanol (10µl/ml) (not provided) and mix by vortexing vigorously.

6. Centrifuge at ≥10,000 rpm for 2 minutes and transfer the supernatant carefully into the new RNase free 1.5ml microcentrifuge tube without disturbing pellet.

7. Add 500µl of ethanol (96 to 100%) mix by pipetting up and down.

8. Transfer upto 700µl 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 700µl. For sample volumes >700µl, simply load the remaining sample, balance the microcentrifuge and spin again. Discard the flowthrough.

9. Place the spin column in the same collection tube. Wash the spin column with 700µl of buffer RW1, 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 15 seconds at ≥10,000rpm. Discard the flowthrough.

11. Place the spin column in the same collection tube. Wash the spin column with 500µl of buffer RW2 by centrifuging for 2 minutes at ≥10,000rpm. Long centrifugation is necessary to remove the wash buffer completely.

12. Place the spin column in a RNase free 1.5ml microcentrifuge tube. 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. Reload the above eluate in the same spin column. Close the lid gently, incubate at room temperature for 1 minute and then centrifuge for 1 minute at ≥10,000 rpm to elute in the same microcentrifuge tube.

14. Protocol (Total RNA purification from Gram Positive Bacteria)

14.1 Important Points Before Starting

1. Prepare water bath or oven at 37 °C
2. Arrange (96-100%) ethanol and commercially available b-mercaptoethanol.
3. Arrange cell wall lysing enzyme eg. lysozyme etc.
4. Arrange Proteinase K (20mg/ml or 600 mAU/ml).
5. All plastic wares and glass wares should be RNase free.
6. Bacterial lysis in this protocol consists of treatment with 20 mg/ml lysozyme and 20 mg/ml or 600 mAU/ml proteinase K along with 15 minutes incubation at 37 °C which is optimal with E. coli.

   Enzymatic lysis is affected by bacterial species, cell number, and culture medium, it may be therefore necessary to adjust enzyme concentration, enzyme incubation time, or to treat with a different enzyme.
7. Add 10 µl of b-mercaptoethanol per 1ml buffer RG and mix well. Buffer RG is stable at room temperature for 1 month after addition of b-mercaptoethanol.

14.2 Procedure

1. Centrifuge 1-3 ml ($5x10^8$ - $7.5x10^8$) cells from an overnight bacterial culture at $\geq$10,000 rpm for 5 minutes. Remove all traces of supernatant by inverting the open tube until all medium has been drained.
2. Add 200µl of buffer TE containing lysozyme (20mg/ml) and add 20µl of Proteinase K from stock 20mg/ml or 600mAU/ml.
3. Resuspend the pellet completely by pipetting up and down. No cell clumps should be visible after resuspension.

4. Incubate at 37 °C for 15 minutes. Mix by inverting the tube for 2-3 times during incubation. Incubation time can be extended without affecting the procedure, and may increase the RNA yield.

5. Add 700 µl of buffer RG containing b-mercaptoethanol (10µl/ml) and mix by vortexing vigorously.

6. Centrifuge at ≥10,000 rpm for 2 minutes and transfer the supernatant carefully into the new RNase free 1.5ml microcentrifuge tube (not provided) without disturbing pellet.

7. Add 500µl of ethanol (96 to 100%) mix by pipetting up and down.

8. Transfer upto 700µl 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 700µl. For sample volumes >700µl, simply load the remaining sample, balance the microcentrifuge and spin again. Discard the flowthrough.

9. Place the spin column in the same collection tube. Wash the spin column with 700µl of buffer RW1, 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 15 seconds at ≥10,000rpm. Discard the flowthrough.

11. Place the spin column in the same collection tube. Wash the spin column with 500µl of buffer RW2 by centrifuging for 2 minutes at ≥10,000rpm. Long centrifugation is necessary to remove the wash buffer completely.

12. Place the spin column in a RNase free 1.5ml microcentrifuge tube. 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. Reload the above eluate in the same spin column. Close the lid gently, incubate at room temperature for 1 minute and then centrifuge for 1 minute at $\geq$10,000 rpm to elute in the same microcentrifuge tube.

15. Trouble Shooting Guide

A. Lysate may contain particulate material after addition of Buffer RG

1. Centrifugation at low speed	Centrifuge the sample after addition of Buffer RG at ≥10,000rpm.
2. Short period of centrifugation	Increase centrifugation period by 2-3 minutes.
3. Use of excess starting material	Repeat the procedure with the correct amount of starting material.
4. Cell Pellet was not completely resuspended	Resuspend the Pellet completely in buffer TE.

B: mdi Mini Spin Column choked

1. Use of excess starting material	Repeat the procedure with the correct amount of starting material.
2. After addition of buffer RG, Lysate may contain particulate material even after centrifugation	Increase centrifugation period by 2-3 minutes.

C: Low RNA Yield

1. Incomplete resuspension of cell pellets	The bacterial pellet should be evenly resuspended in buffer TE by pipetting up and down or by vigorous vortexing.
2. Poor bacterial growth	Inoculate from a freshly streaked plate and incubate in a shaker at proper speed.
3. Poor cell lysis	Optimize the lysozyme concentration and incubation period.

4. Cells were grown past logarithmic phase	Harvest cells during the mid logarithmic growth phase to ensure the highest RNA yields.
5. The amount of starting material was incorrectly calculated	Repeat the procedure using the correct amount of starting material.
6. Improper dispensing of the elution buffer.	The elution must be dispensed properly on to the center of the column.
D: Low quality RNA	
1. Degraded RNA	Use RNase free plastic and glasswares.
E: 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 ≥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.

18. Ordering Information

To order please specify as below:

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

Example:

BMRK	XX	XX	XX	X	0250

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