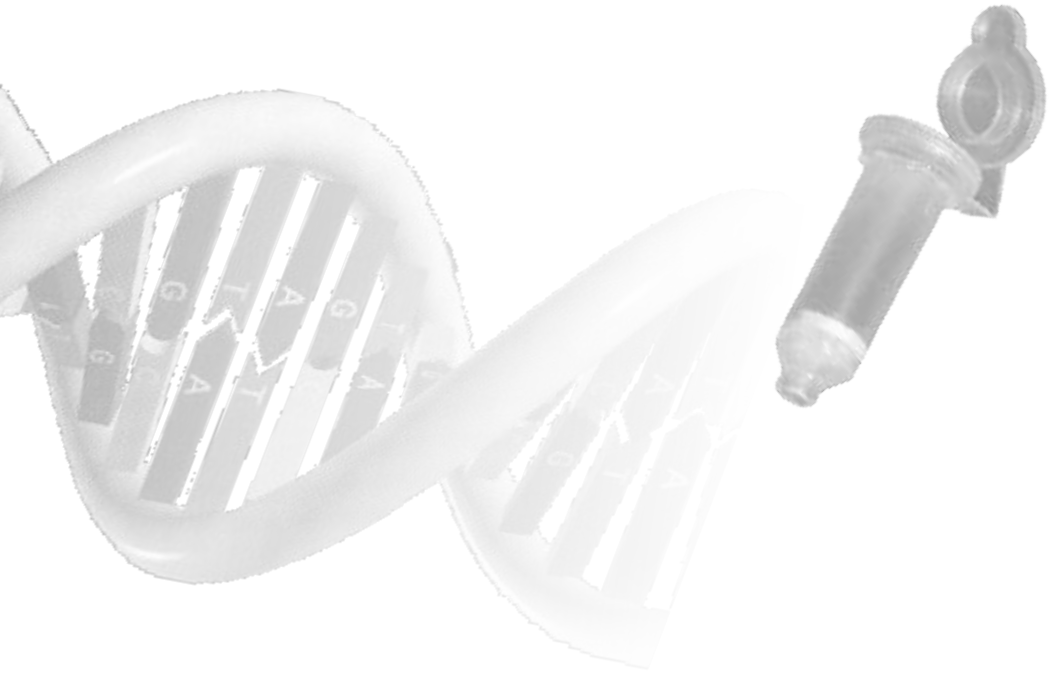
# Plant Total RNA Miniprep Kit



## User Guide

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## 1. Introduction

mdi Plant Total RNA Miniprep Kit is designed to have a fast, easy and economical isolation of upto 100µg of high purity total RNA from plant samples. The mdi Plant 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 plant sample 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 Plant 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 Plant 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 Plant Total RNA Miniprep Kit.

## 6. Lot Release Criteria

Each lot of mdi Plant 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 |
| Shredder Columns | 50 | 250 | 1000 | 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 | 100 | 500 | 2000 | 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

| | |
|---|---|
| Plant Tissue | 100 mg |
| Buffer RG | 450µl |
| Buffer RW1 | 700µl |
| Buffer RW2 | 500µl x 2 |
| Buffer RE | 50µl |

## 11. Handling and Storing Starting Material

RNA in plant tissues is not protected after harvesting until the sample is frozen, or disrupted and homogenized in the presence of denaturing reagents. Unwanted changes in the gene expression profile will occur. It is therefore important that the tissue samples are immediately frozen in liquid nitrogen and stored at -70°C.

Tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization, samples can be stored at -70°C for months.

## 12. Principle

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

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

1. Lysis of Plant Tissue

To efficiently lyse the plant tissue, grind the plant material (maximum 100 mg) under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the powder and liquid nitrogen to an appropriately sized micro-centrifuge tube and allow the liquid nitrogen to evaporate.

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.

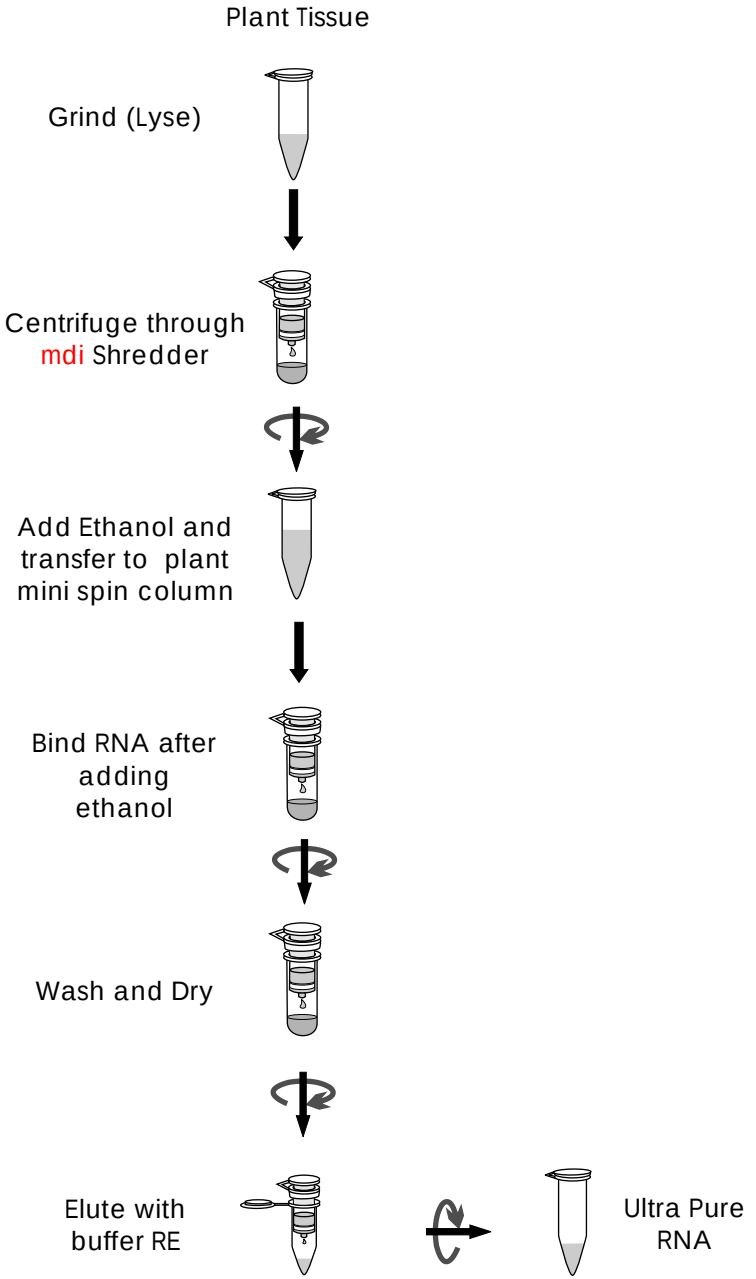
4. Elution

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)

| Plant Sample | Elution Volume | Sample Size (Leaves) | RNA Yield (µg) |
|---|---|---|---|
| Tomato | 50 µl | 100 mg | 73.9 |
| Cauliflower | 50 µl | 100 mg | 63.4 |
| Potato | 50 µl | 100 mg | 38.2 |
| Spinach | 50 µl | 100 mg | 22.5 |
| Carrot | 50 µl | 100 mg | 21.7 |
| Cotton | 100 µl | 100 mg | 11.3 |

## 13. mdi Plant Total RNA Miniprep Procedure



## 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 100 mg plant material can be generally processed.
2. Do not overload the column as this will significantly reduce the RNA yield and quality.
3. Starting material should be efficiently disrupted and homogenized. Disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced RNA yields. Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Incomplete homogenization results in inefficient binding of RNA to the mdi spin column membrane and therefore significantly reduced RNA yields.

| Disruption Method | Homogenization Method |
|---|---|
| Mortar & Pestle | mdi Shredder Spin Column |

4. Arrange (96-100%) ethanol and commercially available b- mercaptoethanol.
5. 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.
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 place at room temperature (15 - 25°C)

## 14.2 Procedure

1. Grind the plant material (maximum 100 mg) under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the powder and liquid nitrogen to an appropriately sized microcentrifuge tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw, proceed immediately to the next step.
2. Add 450µl of buffer RG containing b-mercaptoethanol (10µl/ml) and mix by vortexing vigorously.
3. Transfer the lysate to a mdi shredder spin column placed in a 2 ml collection tube and centrifuge at ≥ 10,000rpm for 2 minutes. Then transfer the supernatant carefully from the collection tube in to the new RNase free 1.5ml microcentrifuge tube without disturbing the pellet.
4. Add 0.5 volume of ethanol (96 - 100%) to the cleared lysate and mix immediately by pipetting. Example: Add 200µl of ethanol in to 400µl of lysate.
5. 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 700µl. For sample volumes >700µl, simply load the remaining sample, balance the microcentrifuge and spin again. Discard the flowthrough.
6. 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.
7. 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.

8. 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.

9. 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.

10. 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.

## 15. Trouble Shooting Guide

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

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|---|---|
| 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. |

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. |
| 3. Incorrect lysate preparation | Grind the plant material under liquid nitrogen to a fine powder. No tissue clump should remain. |
| 4. Lysate was not processed with mdi Shredder Spin Column | Pass the lysate after adding and mixing of Buffer RG through the mdi Shredder Spin Column and use only the supernatant from the collection tube without disturbing the pellet. |

C: Low RNA Yield

| | |
|---|---|
| 1. Use of excess starting material | The plant material should not weigh more than 100 mg. |
| 2. Insufficient disruption of plant sample | Grind the plant material under liquid nitrogen to a fine powder. No tissue clump should remain. |

| | |
|---|---|
| 3. Spin column choked | The column can choke in case the lysate is not clear before loading. Use the lysate after processing with mdi Shredder Spin Column |
| 4. Improper dispensing of elution buffer | The elution buffer must be dispensed properly on to the center of the column membrane. |
| 5. Insufficient incubation of elution buffer in the column membrane. | Increase incubation time by 2 - 3 minutes. |
| D: Low $A_{260}/A_{280}$ value | |
| 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 | |
| Degraded RNA | Use RNase free plastic and glasswares. |
| F: 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. |

## 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 |
| PMRK | PMRK | | | | | 50 | 0050 |
| | | | | | | 250 | 0250 |
| | | | | | | 1000 | 1000 |

Example:

| PMRK | XX | XX | XX | X | 0250 |
|---|---|---|---|---|---|

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