ATP Biotech Inc.

**ATP™ Total RNA Mini Kit (Plant)**
**Catalog No. ARP050/ARP100**

**ATP™ Total RNA Maxi Kit (Plant)**
**Catalog No. ARPM10**

# ATP™ Total RNA Mini Kit (Plant)

Store at room temperature (15-25℃)

## Contents



## Introduction

Format ： Spin column
Sample ： 50 mg plant tissue
Operation ： Centrifuge / vacuum manifold
Operation time ＜ 60 minutes
Yield ： 5-30 μg
Elution volume ： 50 μl
Application ：RT-PCR、Real-Time PCR、Nothern Blotting、mRNA Selection、cDNA Synthesis、Primer Extension

ATP™ Plant Total RNA Mini Kit provides a fast and simple method to isolate total RNA from plant tissue and cells. In the process, sample is first ground in liquid nitrogen and filtrated by filter column to remove cell debris. In the presence of binding buffer with chaotropic salt, the total RNA in the lysate binds to glass fiber matrix in the spin column. The optional DNase treatments can remove DNA residues and the contaminants are washed by wash buffer containing ethanol. Finally, the purified total RNA is eluted by RNase-free water. The protocol does not require phenol extraction and alcohol precipitation.

## Quality Control

The quality of Plant Total RNA Mini Kit was tested on a lot-to-lot basis. The Kits were tested by isolation of total RNA from 25 mg young leaf. Purified RNA could be quantified with spectrophotometer and checked by agarose gel.

## Kit Contents：Cat.No. / Kit Contents

| ARP050 (50 prep/kit ) | ARP100 (100 prep/kit ) |
|---|---|
| RB Buffer ：30 ml | RB Buffer ：60 ml |
| PRB Buffer：30 ml | PRB Buffer：60 ml |
| W1 Buffer ：30 ml | W1 Buffer ：50 ml |
| Wash Buffer (concentrated)*：12.5 ml | Wash Buffer (concentrated)**：25 ml |
| RNase-free water：6 ml | RNase-free water：6 ml |
| RB Columns：50 pcs (yellow/white filter) | RB Columns：100 pcs (yellow/white filter) |
| Filter Column：50 pcs | Filter Column：100 pcs |
| 2 ml Collection Tubes：150 pcs | 2 ml Collection Tubes：300 pcs |

* Add 50 ml ethanol (96~100%) to Wash Buffer prior to initial use.
** Add 100 ml ethanol (96~100%) to Wash Buffer prior to initial use.

**Cautions** : RB Buffer contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

**Use limitation** : For research use only; not for diagnostic or medical purposes

**Equipments and Reagents are provided by User**

- ☐ 1.5 ml microcentrifuge tubes
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ Ethanol (96-100%)
- ☐ DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl ; 20 mM Tris-HCl ; 10 mM $MnCl_2$ ; pH 7.0 at 25℃).

**Protocol Technical Specifications**

Depending on species, plant cells are extremely diverse in their cellular components, such as polysaccharides, lipids, proteins, and other metabolites. According to this characteristic, we provide two different lysis buffers for best performances with various plant tissues.

1. RB Buffer is used in the standard protocol for sample lysis. For most of common plant samples, this buffer system is sufficient to ensure purified RNA with high yields and quality.
2. There is still an alternative buffer, Buffer PRB, provided with the kit. The detergent in this buffer system is more effective in handling plant tissues with large quantities of polysaccharides.

Generally, these two buffer systems could achieve adequate performances. Users may try and chose one best for their demands.



# Total RNA Mini (Plant) Protocol

- ☐ ART050: Add 50 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
  ART100: Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required: sufficient β-mercaptoethanol、Microcentrifuge tube、(96-100%)Ethanol、DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl ; 20 mM Tris-HCl ; 10 mM $MnCl_2$ ; pH 7.0 at 25 ℃).

## Tissue Dissociation

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder. Transfer it into a microcentrifuge tube (provided by user). For some plant sample, we can destruct it without liquid nitrogen.

## Lysis

3. Add 500 µl RB Buffer (or PRB Buffer) and 5 µl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing. And then incubate at room temperature for 5 minates.
4. Place a Filter column in a 2 ml Collection tube. Apply sample mixture to the column.
5. Centrifuge at full speed (13,000 rpm) for 2 minutes.
6. Discard the filter column and transfer the clarified filtrate into a new microcentrifuge tube (provided by user).

## RNA Binding

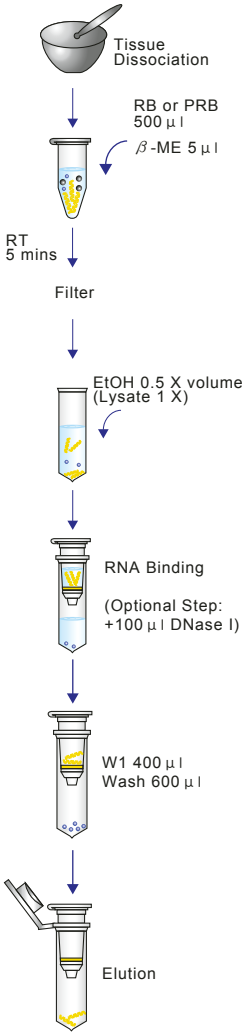
7. Place a RB Column in a 2 ml Collection Tube.
8. Add half sample volume of (96-100%) ethanol to the sample lysate from step 6 and mix immediately by vortexing. (For example, add 250 µl of ethanol to 500 µl of filtrate.)
9. Centrifuge at full speed (13,000 rpm) for 2 minute.
10. Discard the flow-through and place the RB Column back in the Collection Tube.

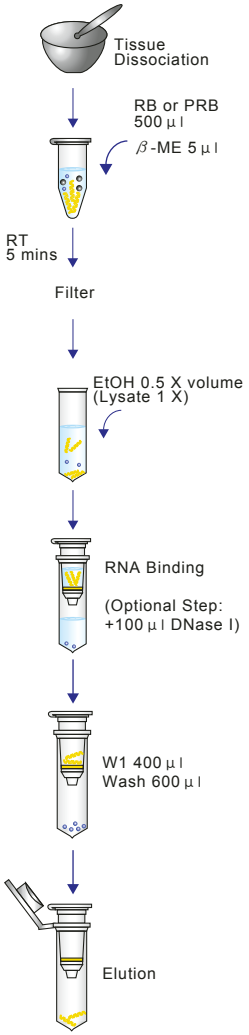
Optional Step: DNA residue degradation

- Add 100 µl DNase I (2 U/µl, provided by user) onto the center of the RB column matrix. Incubate for 10 minutes at room temperature.
- Go to step 11 for Washing procedure



**ATP™ Total RNA Mini DNA (Plant)**






## Washing

11. Add 400 µl of W1 Buffer into the RB column.
12. Centrifuge at 10,000x g (13,000 rpm) for 30 seconds.
13. Discard the flow-through and place the RB Column back in the Collection Tube.
14. Add 600 µl of Wash Buffer (ethanol added) into the RB column.
15. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
16. Discard the flow-through and return the RB Column in the 2ml Collection Tube.
17. Centrifuge again for 3 minutes at full speed to dry the column matrix.

## RNA Elution

18. Transfer dried RB Column in a clean 1.5ml microcentrifuge tube (RNase-free, provided by user).
19. Add 50 µl of RNase-free water into the center of the column matrix.
20. Stand for 3 minutes until Elution Buffer absorbed by the matrix.
21. Centrifuge at 13,000 rpm for 1 minute to elute purified RNA.



# ATP™ Total RNA Maxi Kit (Plant)

Store at room temperature (15-25℃)

## Introduction

Format ： Spin column
Sample ： 500 mg plant tissue
Operation ： Centrifuge / vacuum manifold
Operation time ＜ 60 minutes
Yield ： 50-300 µg
Elution volume ： 50 µl
Application ：RT-PCR、Real-Time PCR、Notthern blotting、mRNA selection、cDNA synthesis、Primer extension

ATP™ Plant Total RNA Mini Kit provides a fast and simple method to isolate total RNA from plant tissue and cells. In the process, sample is first ground in liquid nitrogen and filtrated by filter column to remove cell debris. In the presence of binding buffer with chaotropic salt, the total RNA in the lysate binds to glass fiber matrix in the spin column. The optional DNase treatments can remove DNA residues and the contaminants are washed with an ethanol contained wash buffer. Finally, the purified total RNA is eluted by RNase-free water. The protocol does not require phenol extraction and alcohol precipitation.

## Kit Contents：Cat.No. / Kit Contents

**ARPM010 (10 prep/kit )**

RB Buffer ：60 ml
PRB Buffer：60 ml
W1 Buffer ：50 ml
Wash Buffer (concentrated)*：25 ml
RNase-free water：6 ml
RB Columns：10 pcs
Filter Column：10 pcs

* Add 100 ml ethanol (96~100%) to Wash Buffer prior to initial use.

**Caution**：RB Buffer contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

**Use limitation**：For research use only; not for diagnostic or medical purposes

**Equipments and Reagents are provided by User**

- ☐ 50 ml centrifuge tubes
- ☐ Centrifuge With Swing-Bucket Rotor for 50 ml centrifuge tube
- ☐ Ethanol (96-100%)
- ☐ β-mercaptoethanol
- ☐ DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl；20mM Tris-HCl；10mM $MnCl_2$；pH 7.0 at 25℃).

**Protocol Technical Specifications**

Because of different plant species contain a lot of different metabolites like polysaccharides, polyphenolics, or proteins. Therefore, we provide two different lysis buffers for the various plant samples.

The standard protocol uses RB Buffer for lysis of plant sample. For most of common plant species, the buffer system ensures purified RNA with high yields and a good quality.

Alternatively, Buffer PRB is provided with the kit also. The different detergent in this lysis buffer is suitable for some plant sample with a lot of polysaccharides.



# Total RNA Maxi (Plant) Protocol

- ☐ ARPM10: Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required: sufficient β-mercaptoethanol、50 ml centrifuge tubes、(96-100%)Ethanol、DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl；20mM Tris-HCl；10mM $MnCl_2$；pH 7.0 at 25 ℃).

## Tissue Dissociation

1. Cut off 500 mg (up to 1 g) of fresh or frozen plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder. Transfer it into a 50 ml centrifuge tube (not provided). For some plant sample, we can destruct it without liquid nitrogen.

## Lysis

3. Add 5 ml RB Buffer (or PRB Buffer) and 50 µl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing. Incubation at room temperature for 5 minutes.
4. Place a Maxi Filter column in a 50 ml Centrifuge tube. Apply sample mixture to the column.
5. Centrifuge for 5 minutes at full speed.
6. Discard the Maxi filter column and transfer the clarified filtrate to a new 50 ml centrifuge tube (not provided).

## RNA Binding

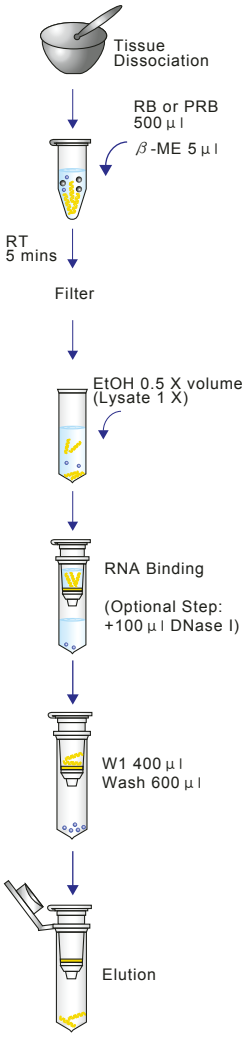
7. Place a Maxi-RB Column in a 50 ml Centrifuge Tube.
8. Add a half of volume of (96-100%) ethanol to the sample lysate from step 6 and mix immediately by vortexing. (For example, add 2.5 ml of ethanol to 5 ml of filtrate.)
9. Centrifuge for 5 minute at full speed.
10. Discard the flow-through and place the Maix-RB Column back in the 50 ml Centrifuge tube.

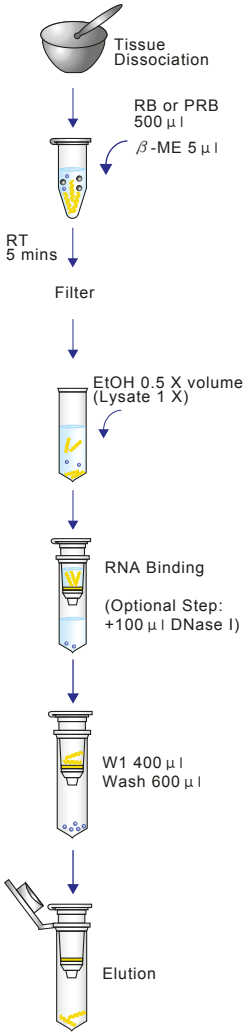
Optional Step: DNA residue degradation

- Add 1 ml DNase I (2 U/µl, not provided) to the center of the RB column matrix. Stand for 10 minutes at room temperature. Incubate at room temperature for 5minutes.
- Go to step 11 for Washing procedure










## Washing

Washing

11. Add 4 ml of W1 Buffer in the Maxi-RB column.
12. Centrifuge for 3 minute at full speed.
13. Discard the flow-through and place the Maxi-RB Column back 50 ml Centrifuge tube.
14. Add 6 ml of Wash Buffer (ethanol added) in the Maxi-RB column.
15. Centrifuge for 3 minute at full speed.
16. Discard the flow-through and return the Maxi-RB Column to the 50 ml Centrifuge tube.
17. Centrifuge again for 10 minutes at full speed to dry the column matrix.

## RNA Elution

18. Transfer dried Maxi-RB Column into a clean 50 ml Centrifuge tube (RNase-free , not provided).
19. Add 500 µl of RNase-free water into the center of the column matrix.
20. Stand for 5 minutes until Elution Buffer absorbed by the matrix.
21. Centrifuge for 5 minute at full speed to elute purified RNA.



# Troubleshooting

| Problem | Possible Reasons / Solution |
|---|---|
| Column clogged | **Sample overloading**<br>・Reduce sample volume or separate into multiple tubes. |
| | **Precipitate was formed at RNA Binding Step**<br>・Reduce the sample material.<br>・Prior to loading the column, break up precipitate in ethanol-added lysate.<br>・It is better for centrifugation at room temperature than 4℃. |
| Low yield | **Insufficient disruption and homogenization**<br>・Grind the sample under liquid nitrogen to a fine powder indeed.<br>・Indeed mix powder of samples with RB/PRB and β-mercaptoethanol well by vortexing. |
| | **Incomplete RNA Elution**<br>・Elute twice to increase yield. |
| RNA degraded | **Harvested samples were not immediately stabilized**<br>・Make sure that harvested samples were stabilized immediately.<br>・It is better to use fresh samples than frozen samples. |
| | **RNase contamination**<br>・I Make sure all centrifuge tubes and tips used in RNA extraction are RNase-free. |

If the lysate mixture could not flow past membrane spin column after centrifugation, increase the centrifuge time until the lysate mixture passes completely.

### Reference

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76,615.

## Note

## Catalog

| Product Name | Package | Cat. No. |
|---|---|---|
| ATP™ Plasmid Mini Kit | 100/300 prep | APD100/APD300 |
| ATP™ Plasmid Midi Kit (Ultra Pure) | 25 prep | API25 |
| ATP™ Plasmid Maxi Kit (Ultra Pure) | 10/25 prep | APM10/APM25 |
| ATP™ 96-Well Plasmid Mini Kit | 4/10 plates | APD9604/APD9610 |
| ATP™ Plasmid Mini Binding Column | 50 pcs | PDC50 |
| ATP™ Plasmid Midi Resin Column | 10 pcs | PIC10 |
| ATP™ Plasmid Maxi Resin Column | 10 pcs | PMC10 |
| ATP™ Gel/PCR DNA Fragments Extraction Kit | 100/300 prep | ADF100/ADF300 |
| ATP™ 96-Well Gel/PCR DAN Extraction Kit | 4/10 plates | ADF9604/ADF9610 |
| ATP™ 96-Well SEQ Dye Clean Up Kit | 4/10 plates | ADC9604/ADC9610 |
| ATP™ Fragment DNA Binding Column | 50 pcs | DFC50 |
| ATP™ Genomic DNA Mini Kit (Blood/Culture Cell/Bacteria) | 100/300 preps | AGB100/AGB300 |
| ATP™ Genomic DNA Mini Kit (Tissue) | 50/300 preps | AGT050/AGT300 |
| ATP™ Genomic DNA Mini Kit (Plant) | 100 preps | AGP100 |
| ATP™ Genomic DNA Maxi Kit (Fresh Blood) | 25 prep | AGBM25 |
| ATP™™ Genomic DNA Maxi Kit (Frozen Blood) | 25 prep | AGDM25 |
| ATP™ Plant Genomic DNA Maxi Kit | 25 prep | AGPM25 |
| ATP™ 96-Well Genomic DNA Kit | 4/10 plates | AGB9604/AGB9610 |
| ATP™ Reagent Genomic DNA Kit | For 100ml blood | AGE100 |
| ATP™ Genomic DNA Binding Column | 50 pcs | GDC50 |
| ATP™ RNA Mini Kit (Blood/Culture Cell/Bacteria) | 50 prep | ARB050 |
| ATP™ RNA Mini Kit (Tissue) | 50 prep | ART050 |
| ATP™ RNA Mini Kit (Plant) | 50 prep | ARP050 |
| ATP™ Viral Nucleic Acid Mini Kit | 50 prep | AVR050 |
| ATP™ 96-Well Viral Nucleic Acid Kit | 4/10 plates | AVR9604/AVR9610 |
| ATP™ RNA Maxi Kit | 10 prep | ARTM10 |
| ATP™ Plant RNA Maxi Kit | 10 prep | ARPM10 |
| ATP™ RNA Binding Column | 50 pcs | RBC50 |
| Proteinase K | 11 mg/kit | APK000011 |
| RNase A | 0.2 ml (50 mg/ml) | ARA500200 |
| RNase A | 1.5 ml (50 mg/ml) | ARA501500 |


www.atp-biotech.com