ATP™ Total RNA Mini Kit (Tissue)

Store at room temperature (15~25℃)

Introduction

Format ： Spin column
Sample ： Up to 25 mg of tissue
Operation ： Centrifuge / vacuum manifold
Recovry ： Up to 25 μg
Elution volume ： 50 μl
Application ：RT-PCR、Real-time PCR、Notthern blotting、mRNA selection、cDNA synthesis、Primer extension

ATP™ Total RNA Mini Kit (Tissue) is specially designed for purification of total RNA from a variety of animal tissues or cells. The provided micropestle can efficiently homogenize tissue samples in the microcentrifuge tube. The method uses detergent and a chaotropic salt to lyse cells and inactivate RNase. Then RNA in the chaotropic salt is bonded to the glass fiber matrix of the column. After washing off the contaminants, the purified RNA is eluted by RNase-free water. The entire procedure can be completed in 20 minutes and the purified RNA is ready for RT-PCR, Northern blotting, primer extension and cDNA library construction.

Kit Contents：Cat.No. / Kit Contents

ART50 (50prep/kit)		ART100 (100 prep/kit)	
RB Buffer	：30 ml	RB 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：100pcs		2 ml Collection Tubes：200pcs	
Micropestle ：50pcs		Micropestle ：100pcs	

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

Recommend

- ☐ For High-throughput sample processing, ATPTM 96-Well Genomic DNA Kit is recommended.
- ☐ For purification of viral RNA, the ATPTM Nucleic Acid Extraction Kit is recommended. All buffers and components of this kit are guaranteed to be RNase free.

Equipments and Reagents are provided by User

- ☐ 1.5 ml microcentrifuge tubes
- ☐ Microcentrifuge (with rotor for 2 ml tubes)
- ☐ Ethanol (96-100%)
- ☐ Ethanol (70%)
- ☐ 20-G needle syringe
- ☐ DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl ; 20mM Tris-HCl ; 10mM $MnCl_2$; pH 7.0 at 25℃).

Total RNA Mini (Tissue) Protocol

Equipments and Reagents are provided by User

- ☐ 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: 20-G needle syringe、Microcentrifuge tube、Ethanol (70%)、DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl；20mM Tris-HCl；10mM $MnCl_2$；pH 7.0 at 25 ℃).

Cell Lysis

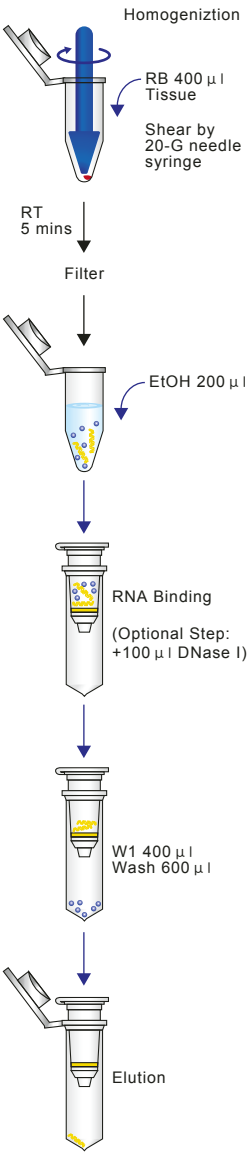
1. Cut up to 10mg of fresh or frozen animal tissue and transfer it into a microcentrifuge tube (not provided).
2. Add 400 µl RB Buffer into the tube and use provided Micropestle to grind the tissue a few times.
3. Shear the tissue by passing lysate through a 20-G needle syringe 10 times.
4. Incubate at room temperature for 5 minutes.
5. Place a Filter Column in a 2 ml Collection Tube. Apply sample mixture to the column.
6. Centrifuge for 2 minutes at full speed (13,000 rpm).
7. Discard the filter column and transfer the clarified filtrate to a new microcentrifuge tube (not provided).

RNA Binding

8. Place a RB Column in a 2 ml Collection Tube.
9. Add 400 µl of 70% ethanol to the sample lysate from step 7 and mix immediately by pipetting.
10. Centrifuge at full speed (13,000 rpm) for 2 minute.
11. 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, 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 12 for Washing procedure



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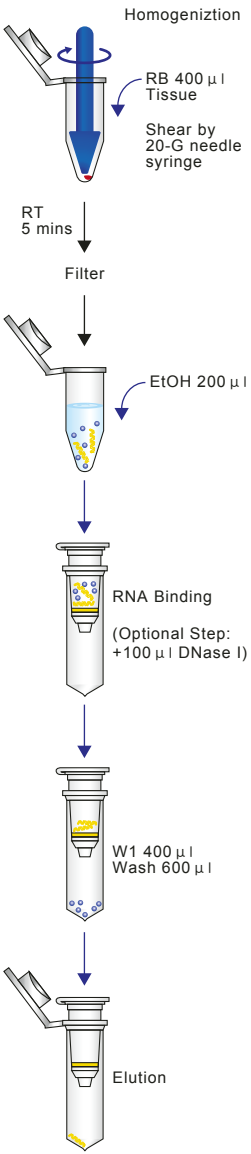

Washing

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

RNA Elution

19. Transfer dried RB Column into a clean 1.5ml microcentrifuge tube (RNase-free，not provided).
20. Add 50 µl of RNase-free water into the center of the column matrix.
21. Stand for 3 minutes until Elution Buffer absorbed by the matrix.
22. Centrifuge at 13,000 rpm for 1 minute to elute purified RNA.

Troubleshooting

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

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 Binding Column	50 pcs	PDC50
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™ Reagent Genomic DNA Kit	For 100ml blood	AGE100
ATP™ Genomic DNA Mini Kit (Blood/Culture Cell)	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)	10 prep	AGBM10
ATP™™ Genomic DNA Maxi Kit (Frozen Blood)	10 prep	AGDM10
ATP™ Plant Genomic DNA Maxi Kit	10 prep	AGPM10
ATP™ 96-Well Genomic DNA Kit	4/10 plates	AGB9604/AGB9610
ATP™ Genomic DNA Binding Column	50 pcs	GDC50
ATP™ RNA Mini Kit (Blood/Culture Cell)	50prep	ARB050
ATP™ RNA Mini Kit (Tissue)	50prep	ART050
ATP™ RNA Mini Kit (Plant)	50prep	ARP050
ATP™ Viral Nucleic Acid Extraction Kit	50prep	AVR050
ATP™ Viral Nucleic Acid 96-Well 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