ATP™ Total RNA Mini Kit (Blood/Culture Cell/Bacteria)

Store at room temperature (15~25℃)

Introduction

Format ： Spin columns
Sample ： Up to 300 μl of whole blood
Up to 10^6 animal cultured cells
Up to 10^8 bacterial cultured cells
Operation ： Centrifuge / vacuum manifold
Operation time ： 20 minutes
Yield ： Up to 30 μg
Application ： RT-PCR、Real-time PCR、Notthern blotting、mRNA selection、cDNA synthesis、Primer extension

ATP™ RNA Mini Kit (Blood/Culture cell) is specially designed for purification of total DNA (including: genomic, mitochondrial and viral DNA) from fresh whole human blood, plasma, serum, buffy coat, other body fluids, lymphoctes, bacteria and cultured cells. This method uses detergents and a chaotropic salt to lyse cells and inactivate RNase, and then RNA in 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.

Kit Contents：Cat.No. / Kit Contents

ARB50 (50 preps/kit)	ARB100 (100 preps/kit)
RBC lysis Buffer：100 ml	RBC lysis Buffer：200 ml
RT Buffer ：15 ml	RT Buffer ：30 ml
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)**：25ml
RNase-free water：6 ml	RNase-free water：6 ml
Filter Column：50 pcs	Filter Column：100 pcs
RB Column：50 pcs (yellow/white filter)	RB Column：100 pcs (yellow/white filter)
2 ml Collection Tubes：100pcs	2 ml Collection Tubes：200pcs

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

Caution : RB Buffer contain guanidine hydrochloride which harmful and irritant agent.
During operation, always wear a lab coat, disposable gloves, and protective goggles.
For more information, please refer to the appropriate material safety data sheets (MSDS).

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

Recommend

1. For larger volumes of whole blood or cultured cells, ATPTM Total RNA Maxi Kits (Blood/Culture cell) is recommended.
2. For High-throughput sample processing, ATPTM 96-Well Total RNA Kit is recommended.
3. 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. 1.5 ml microcentrifuge tubes
2. Microcentrifuge (with rotor for 2 ml tubes)
3. PBS (phosphate-buffered saline) may be required for some samples
4. Ethanol (96-100%)
5. Ethanol (70%)
6. DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl ; 20mM Tris-HCl ; 10mM $MnCl_2$; pH 7.0 at 25℃).
7 Lysozyme Buffer (20 mg/ml lysoyme ; 20 mM Tris-HCl ; 2 mM EDTA ; 1% Triton X-100 , pH 8.0) for Gram-negative bacteria prelysis. Prepare the lysozyme buffer fresh just before use.

Total RNA Mini (Blood/Culture Cell) Protocol

Equipments and Reagents are provided by User

- ☐ ARB050：Add 50 ml ethanol (96-100%) to Wash Buffer prior to the initial use. ARB100：Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required： Microcentrifuge tube、70% 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 ℃).

RBC Lysis / Cell Harvesting

Use fresh human blood:

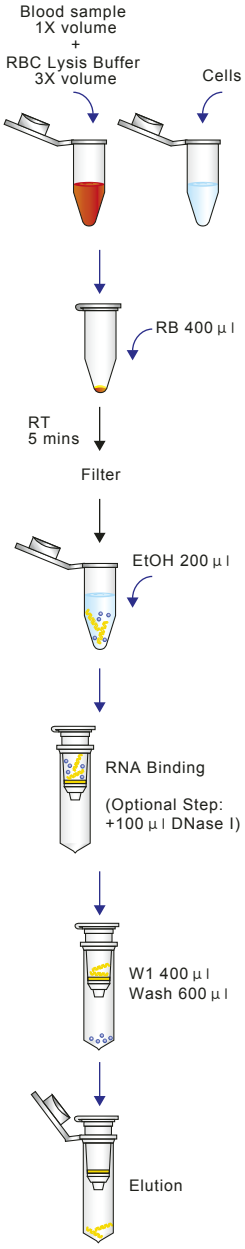
1. Collected fresh blood in EDTA-NA2-treated collection tubes (or other anticoagulant mixtures).
2. Add 1 ml RBC Lysis Buffer to a sterile 1.5 ml reaction tube, and then add 300 µl human whole blood and mix by inversion. Do not vortex. Incubate the tube for 5 min at room temperature.
3. Centrifuge for 2 min at 2500 rpm (500 µg) and discard the supernatant, then add 100 µl RBC Lysis Buffer to resuspend the cell pellet.

Use culture cell:

1. If using adherent cells, trypsinize the cells before harvesting.
2. Transfer 10^6-10^7 of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation for 20 seconds at 6,000 x g (8,000 rpm).
3. Discard the supernatant and resuspend the cells in 100 µl PBS or RBC Lysis Buffer.

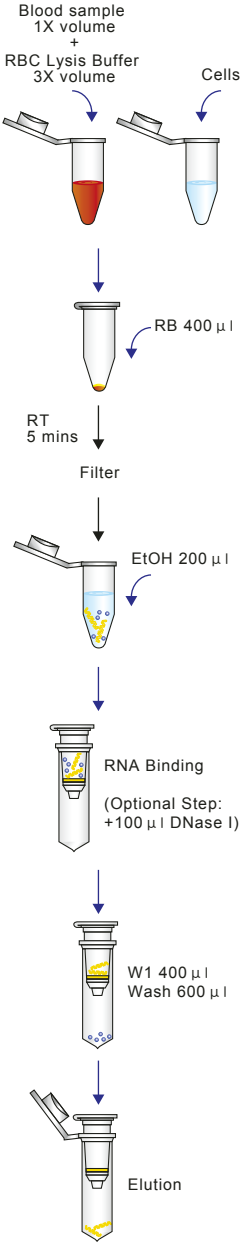
Cell Lysis

4. Add 400 µl RB Buffer to the tube and mix with white pellet (from step 3) by vortexing.
5. Incubate at room temperature for 5 minutes
6. Place a Filter column in a 2 ml Collection tube. Apply sample mixture to the column.
7. Centrifuge for 2 minutes at full speed (13,000 rpm).
8. Discard the Filter column and transfer the clarified filtrate to a new microcentrifuge tube (not provided).



ATP™ Total RNA Mini Kit (Blood/Culture Cell/Bacteria)

Blood / Culture Cell Protocol



RNA Binding

9. Place a RB Column on a 2 ml Collection Tube.
10. Add 400 µl of 70% ethanol to the sample lysate from step 8 and mix immediately by pipetting.
11. Apply 500 µl of ethanol-added mixture from previous step to the RB column.
12. Centrifuge at full speed (13,000 rpm) for 2 minute.
13. Discard the flow-through and apply the rest to the same Column.
14. Centrifuge at full speed (13,000 rpm) for 2 minute.
15. 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 step16. for Washing procedure

Washing

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

RNA Elution

22. Transfer dried RB Column into a clean 1.5ml microcentrifuge tube (RNase-free，not provided).
23. Add 50 µl of preheated RNase-free water into the center of the column matrix.
24. Stand for 3-5 minutes until water has been absorbed by the matrix.
25. Centrifuge at 13,000 rpm for 1 minute to elute purified RNA.

Total RNA Mini (Bacteria) Protocol

☐ ARB050 : Add 50ml ethanol (96-100%) to Wash Buffer prior to the initial use.
ARB100 : Add 100ml ethanol (96-100%) to Wash Buffer prior to the initial use.

☐ Additionally required : Microcentrifuge tube、70% Ethanol、DNase I (2 KU/ml, RNase-free) in reaction buffer (1 M NaCl ; 20mM Tris-HCl ; 10mM MnCl2 ; pH 7.0 at 25 ℃).
For Gram-negative bacteria : RT buffer
For Gram-positive bacteria : Lysozyme Buffer (20 mg/ml lysoyme ; 20 mM Tris-HCl ; 2 mM EDTA ; 1% Triton X-100 , pH 8.0). Prepare the lysozyme buffer fresh just before use.

Cell harvest

For Gram-negative bacteria

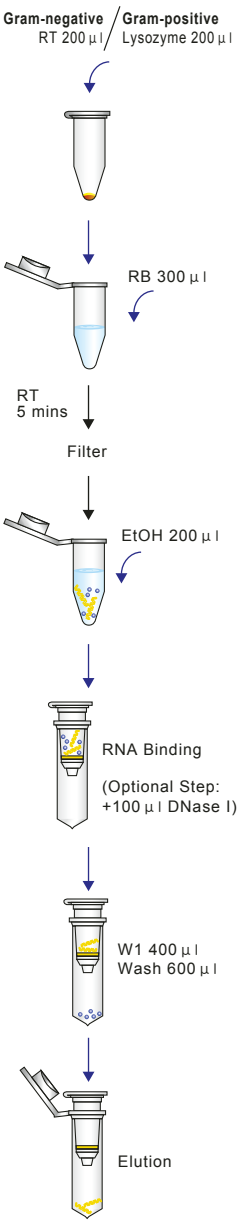
1. Transfer bacterial culture (< 109) to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (13,000 rpm) in a microcentrifuge and discard the supernatant, and then vortexing the cell pellet for 30 seconds.
3. Add 200 µl of RT Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 5 minutes.

For Gram-positive bacteria

1. Transfer bacterial culture (< 109) to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (13,000 rpm) in a microcentrifuge and discard the supernatant.
3. Add 200 µl of Lysozyme Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3min.

Cell Lysis

5. Add 300 µl RB Buffer to the tube and mix with sample lysate (from step 4) by vortexing.
6. Incubate at room temperature for 5 minutes
7. Centrifuge for 2 minutes at full speed (13,000 rpm).
8. Transfer the supernatant to a new microcentrifuge tube.



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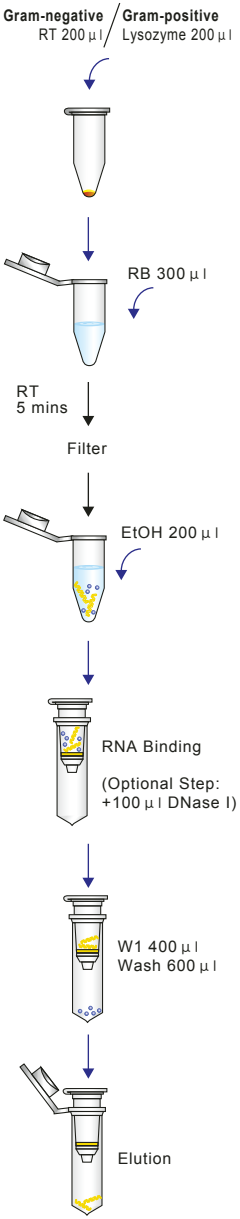
Bacterial Protocol



RNA Binding

9. Place a RB Column on a 2 ml Collection Tube.
10. Add 300 µl of 70% ethanol to the sample lysate from step 8 and mix immediately by pipetting.
11. Apply 500 µl of ethanol-added mixture from previous step to the RB column.
12. Centrifuge at full speed (13,000 rpm) for 2 minute.
13. Discard the flow-through and apply the rest to the same Column.
14. Centrifuge at full speed (13,000 rpm) for 2 minute.
15. 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 step16.to Washing.

Washing

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

RNA Elution

22. Transfer dried RB Column into a clean 1.5ml microcentrifuge tube (RNase-free，not provided).
23. Add 50 µl of preheated RNase-free water into the center of the column matrix.
24. Stand for 3-5 minutes until water has been absorbed by the matrix.
25. 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