ATP Biotech Inc.

ATP™ Total RNA Mini Kit (Blood/Culture Cell/Bacteria)
Catalog No. ARB050/ARB100

ATP™ Total RNA Mini Kit (Tissue)
Catalog No. ART050/ART100

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

Store at room temperature (15~25℃)

Contents

Introduction

Format ： Spin column
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、Nothern Blotting、mRNA Selection、cDNA Synthesis、Primer Extension

ATP™ RNA Mini Kit (Blood/Culture cell) is specially designed for purification of total RNA from fresh whole human blood 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. 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

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)**：25 ml
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：100 pcs	2 ml Collection Tubes：200 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 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

Recommendations

1. For larger volumes of whole blood or cultured cells, ATP™ Total RNA Maxi Kits (Blood/Culture cell) is recommended.
2. For High-throughput sample processing, ATP™ 96-Well Total RNA Kit is recommended.
3. For purification of viral RNA, the ATP™ 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 (RNase-free)
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ β-Mercaptoethanol (β-ME；10 μl of β-ME per 1ml RB Buffer)
- ☐ PBS (phosphate-buffered saline) may be required for some samples
- ☐ Ethanol (96-100%)
- ☐ 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℃).
- ☐ 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.

Notice

We recommend to apply highly purified DNase I (RNase-free) for sensitive downstream applicapions. If genomic DNA contamination may be negligible or inconsequential to the application, DNase I may not be necessary to apply.

Total RNA Mini (Blood/Culture Cell) Protocol

- ☐ 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.
- ☐ Add 10 μl of β-Mercaptoethanol per 1ml of RB Buffer to RB Buffer before use.
- ☐ Additionally required： Microcentrifuge tube、70% 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 ℃).

RBC Lysis / Cell Harvesting

Use fresh human blood:

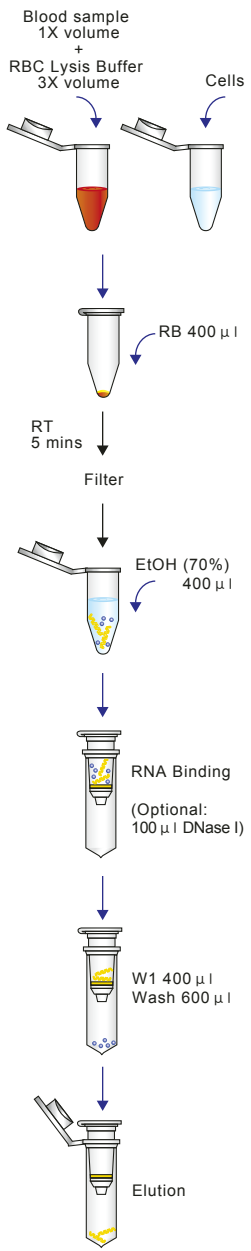
1. Collected fresh blood in EDTA-NA_2-treated collection tubes (or other anti-coagulant mixtures).
2. Add 1 ml RBC Lysis Buffer into a sterile 1.5 ml reaction tube, and then add 300 μl human whole blood and mix by inversion. Do not vortex. Incubate the tube at room temperature for 5 min.
3. Centrifuge at 2500 rpm (500 xg) for 2 min 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 into a microcentrifuge tube (provided by user) 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.

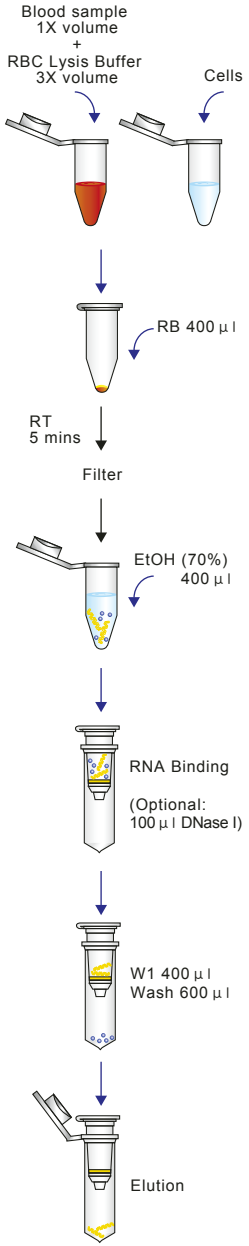
Cell Lysis

4. Add 400 μl of 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 into the column.
7. Centrifuge at full speed (13,000 rpm) for 2 minutes.
8. Discard the Filter column and transfer the clarified filtrate in a new microcentrifuge tube (provided by user).



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

Blood / Culture Cell Protocol



RNA Binding

9. Place a RB Column in 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 into the RB column.
12. Centrifuge at full speed (13,000 rpm) for 2 minute.
13. Discard the flow-through and apply the rest into 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, provided by user) onto the center of the RB column matrix. Incubate for 10 minutes at room temperature.
- Go to step 16 for Washing procedure

Washing

16. Add 400 µl of W1 Buffer into the RB column. Centrifuge at 10,000 xg (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 in 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 in a clean 1.5ml microcentrifuge tube (RNase-free, provided by user).
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 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.
Add 10 µl of β-Mercaptoethanol per 1ml of RB Buffer to RB Buffer before use.

☐ Additionally required： Microcentrifuge tube、70% 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 ℃).

☐ 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 (< 10^9) into a microcentrifuge tube (provided by user).
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

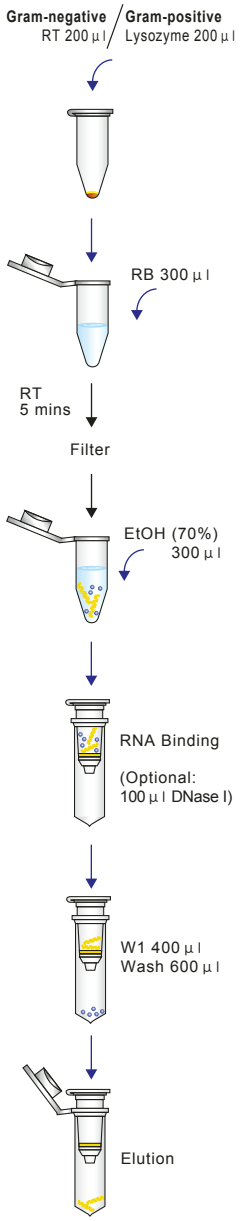
1. Transfer bacterial culture (< 10^9) into a microcentrifuge tube (provided by user).
2. Centrifuge at full speed (13,000 rpm) for 1 min and discard the supernatant.
3. Add 200 µl of Lysozyme Buffer into the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature 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 at full speed (13,000 rpm) for 2 minutes.
8. Transfer the supernatant into a new microcentrifuge tube.

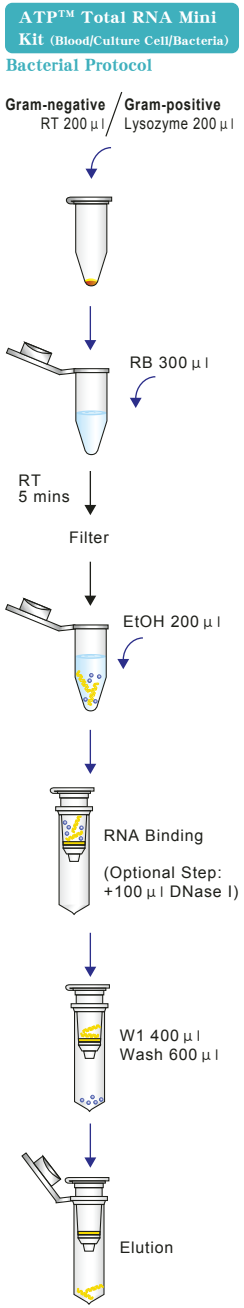
ATP™ Total RNA Mini Kit (Blood/Culture Cell/Bacteria)

Bacterial Protocol



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

Store at room temperature (15~25℃)



RNA Binding

9. Place a RB Column in 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 into the RB column.
12. Centrifuge at full speed (13,000 rpm) for 2 minute.
13. Discard the flow-through and apply the rest into 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, provided by user) onto the center of the RB column matrix. Incubate for 10 minutes at room temperature.
- Go to step 16 for Washing procedure

Washing

16. Add 400 µl of W1 Buffer into the RB column. Centrifuge at 10,000 xg (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 in 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 in a clean 1.5ml microcentrifuge tube (RNase-free, provided by user).
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.

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、Nothern 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 (50 prep/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：150 pcs		2 ml Collection Tubes：300 pcs	
Micropestle ：50 pcs		Micropestle ：100 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.

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

Recommendations

- ☐ For High-throughput sample processing, ATP™ 96-Well Genomic DNA Kit is recommended.
- ☐ For purification of viral RNA, the ATP™ 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 (RNase-free)
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ β-Mercaptoethanol (β-ME；10 μl of β-ME per 1ml RB Buffer)
- ☐ 20-G needle syringe
- ☐ Ethanol (96-100%)
- ☐ Ethanol (70%)
- ☐ 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℃).

Total RNA Mini (Tissue) Protocol

- ☐ 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.
- ☐ Add 10 μl of β-Mercaptoethanol per 1ml of RB Buffer to RB Buffer before use.
- ☐ Additionally required： Microcentrifuge tube、70% 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 ℃).

Cell Lysis

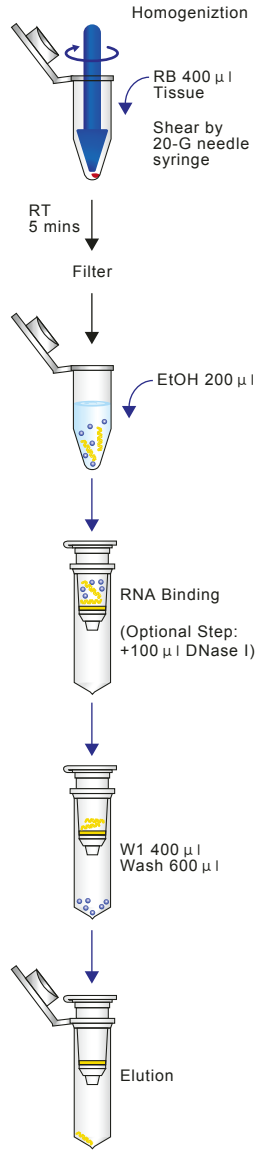
1. Cut up to 10 mg of fresh or frozen animal tissue and transfer it into a micro-centrifuge tube (provided by user).
2. Add 400 μl RB Buffer into the tube and use provided Micropestle to grind the tissue a few times.
3. Shear the tissue by passing lysate through a 20-G needle syringe 10 times.
4. Incubate at room temperature for 5 minutes.
5. Place a Filter Column in a 2 ml Collection Tube. Apply sample mixture into the column.
6. Centrifuge at full speed (13,000 rpm) for 2 minutes.
7. Discard the filter column and transfer the clarified filtrate into a new micro-centrifuge tube (provided by user).

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.

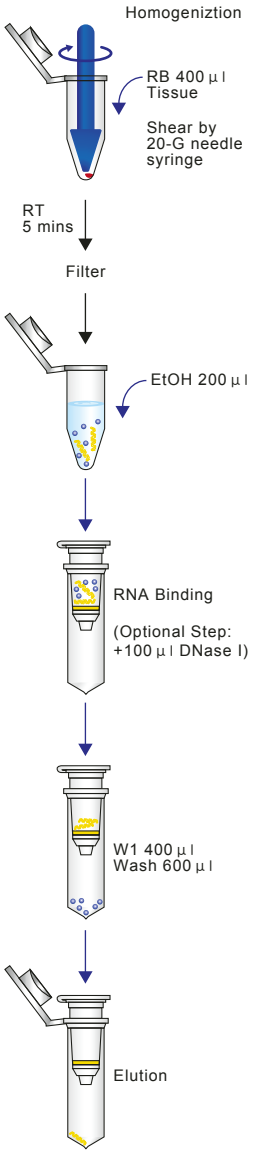
Optional Step: DNA residue degradation

- Add 100 μl DNase I (2 U/μl, provided by user) onto the center of the RB column matrix. Incubate for 10 minutes at room temperature.
- Go to step 12 for Washing procedure



ATP™ Total RNA Mini Kit (Tissue)

Store at room temperature (15~25℃)



Washing

12. Add 400 µl of W1 Buffer into 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 in 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 in a clean 1.5ml microcentrifuge tube (RNase-free, provided by user).
20. Add 50 µl of RNase-free water into the center of the column matrix.
21. Stand for 3 minutes until Elution Buffer has been 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.

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

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