ATP Biotech Inc.

ATP™ Total RNA Maxi Kit (Blood/Culture Cell/Bacteria/Tissue)
Catalog No. ARBM10

ATP™ Total RNA Maxi Kit (Blood/Culture cell/Bacteria/Tissue)

Store at room temperature (15~25℃)

Contents

Introduction

Format ： Maxi Spin column
Sample ： 100-200 mg animal tissue
10^7-10^8 cultured cell
5 ml blood sample
Operation ： Centrifuge
Operation time ＜ 60 minutes
Elution volume ： 500 µl
Yield ： 500 µg
Application ： RT-PCR、Real-Time PCR、Nothern Blotting、mRNA Selection、cDNA Synthesis、Primer Extension

ATP™ Total RNA Maxi Kit provides a fast and simple method to isolate total RNA from animal tissue and cells. In the process, sample is destroyed by grinding 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. The entire procedure can be completed in 60 minutes.

Kit Contents：Cat.No. / Kit Contents

ARBM10 (10 preps/kit)	
RBC Lysis Buffer	200 ml
RT Buffer	30 ml
RB Buffer	60 ml
W1 Buffer	50 ml
Wash Buffer (concentrated)*	25 ml
RNase-free water	6 ml
Filter Column	10 pcs
RB-Maxi Columns	10 pcs

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

Cautions：RB Buffer contain guanidine hydrochloride which is a harmful and 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

- ☐ 15 ml centrifuge tubes (RNase-free)
- ☐ 50 ml centrifuge tubes (RNase-free)
- ☐ Centrifuge With Swing-Bucket Rotor for 50 ml centrifuge tube
- ☐ Ethanol (96-100%)
- ☐ β-Mercaptoethanol (β-ME；10 μl of β-ME per 1ml RB Buffer)
- ☐ DNase I (2 KU/ml, RNase-free; optional) in reaction buffer (1 M NaCl；20mM Tris-HCl；10mM MnCl2；pH 7.0 at 25 ℃).
- ☐ PBS (phosphate-buffered saline) may be required for samples
- ☐ 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 just before use
- ☐ Ice

Notice

For best yield, Centrifuge With Swing-Bucket Rotor is recommened for following protocol. And after Lysis, all steps of this protocol should be performed as quickly as possible.

Total RNA Maxi (Blood) Protocol

- ☐ Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required：15 ml centrifuge tube、50 ml centrifuge tube、96-100 % Ethanol、sufficient β-mercaptoethanol、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

1. Collected fresh blood in EDTA-NA_2-treated collection tubes (or other anti-coagulant mixtures).
2. Apply up to 5 ml of blood into a 50 ml centrifuge tube.
3. Add 3 volumes of RBC Lysis Buffer to 1 volume of the blood sample and mix by inversion. Do not vortex. (For example, add 15 ml of RBC Lysis Buffer to 5 ml blood sample.)
4. Incubate the tube for 10 min on ice.
5. Centrifuge at 500 xg for 5 min at 4℃ and discard the supernatant.

Cell Lysis

6. Add 5 ml of RB Buffer and 50 μl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing.
7. Place a Filter column in a 50 ml centrifuge tube. Apply sample mixture into the column.
8. Centrifuge at full speed for 5 minutes.
9. Discard the filter column and transfer the clarified filtrate to a new 15 ml centrifuge tube (provided by user).

RNA Binding

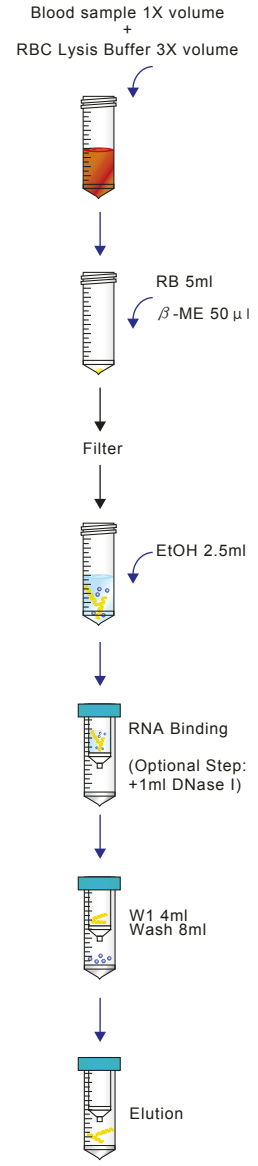
10. Place a RB-Maxi column in a 50 ml centrifuge tube.
11. Add half sample volume of (96-100 %) ethanol to the sample lysate from step 9 and mix immediately by vortexing. (For example, add 2.5 ml of ethanol to 5 ml of filtrate.)
12. Apply ethanol-added mixture from previous step into the RB-Maxi column.
13. Centrifuge at full speed for 5 minutes and discard the flow-through.

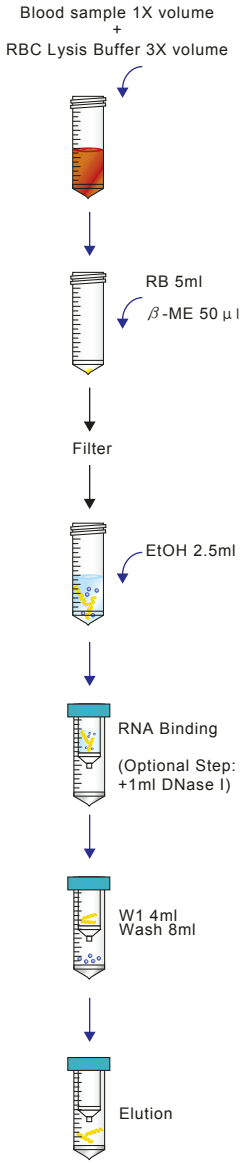
Optional Step: DNA residue degradation

- Add 1 ml DNase I (2 U/μl, provided by user) onto the center of the RB-Maxi column matrix. Incubate for 10 minutes at room temperature.
- Go to step 14 for Washing procedure.

ATP™ Total RNA Maxi

For Fresh Blood





Washing

14. Add 4 ml of W1 Buffer into the RB-Maxi column.
15. Centrifuge at full speed for 3 minutes.
16. Add 8 ml of Wash Buffer (ethanol added) into the RB-Maxi Column.
17. Centrifuge at full speed for 3 minutes.
18. Discard the flow-through and return the RB-Maxi Column in the 50 ml Centrifuge Tube.
19. Centrifuge again for 10 minutes at full speed to dry the column matrix.

RNA Elution

20. Place dried RB-Maxi column in a clean 50 ml centrifuge tube (RNase-free, provided by user).
21. Apply 500 µl of RNase-free water onto the center of the column matrix.
22. Stand for 5 minutes until water has been absorbed by the matrix.
23. Centrifuge at full speed for 5 minute to elute purified RNA.

Total RNA Maxi (Culture Cell) Protocol

☐ Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
☐ Additionally required：15 ml centrifuge tube、50 ml centrifuge tube、96-100 % Ethanol、sufficient β-mercaptoethanol、DNase I (2 KU/ml, RNase-free; optional) in reaction buffer (1 M NaCl；20 mM Tris-HCl；10 mM $MnCl_2$；pH 7.0 at 25 ℃).

Cell Lysis

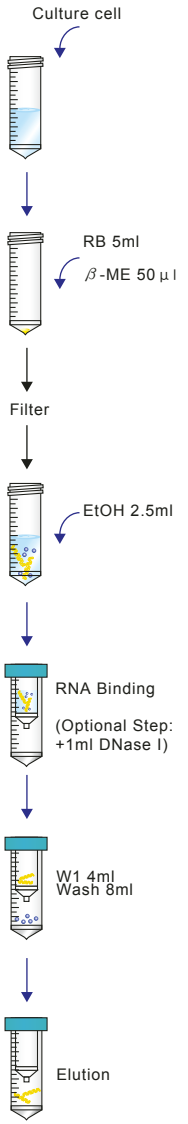
Suspension cultured animal cells：

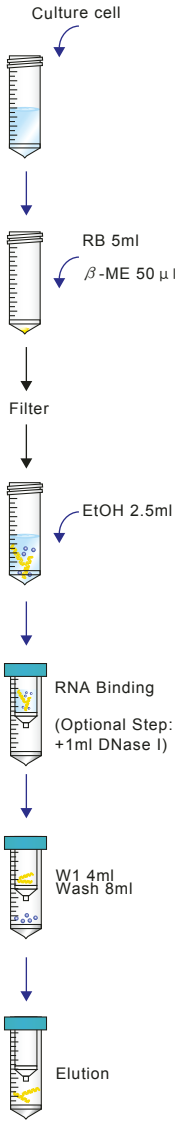
1. Culture cells are in medium.
2. Transfer 10^7-10^8 of cells into a 15 ml centrifuge tube (provided by user) and harvest the cells with centrifugation at 300 xg for 5 minutes.
3. Remove the supernatant.
4. Add 5 ml of RB Buffer and 50 µl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing.
5. Place a Filter column in a 50 ml centrifuge tube. Apply sample mixture into the column.
6. Centrifuge at full speed for 5 minutes.
7. Discard the filter column and transfer the clarified filtrate to a new 15 ml centrifuge tube (provided by user).

Adherent cultured cells： (If use adherent cultured cells, trypsinize cells, before lysis or lyse cells directly in culture dish.)

To trypsinize cells：

1. Remove medium, and wash cells with PBS. Then aspirate PBS and add 0.10-0.25 % trypsin in PBS to trypsinize the cells. After cells detached, add medium and transfer cells to 15 ml centrifuge tube (provided by user).
2. Transfer 10^7-10^8 of cells into a 15 ml centrifuge tube (provided by user) and harvest the cells with centrifugation at 300 xg for 5 minutes.
3. Remove the supernatant.
4. Add 5 ml of RB Buffer and 50 µl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing.
5. Place a Filter column in a 50 ml centrifuge tube. Apply sample mixture into the column.
6. Centrifuge at full speed for 5 minutes.
7. Discard the filter column and transfer the clarified filtrate to a new 15 ml centrifuge tube (provided by user).





To lyse cells in culture dish or flask：

1. Remove culture medium.
2. Add 5 ml of RB Buffer and 50 µl of β-mercaptoethanol (provided by user) into culture dish or flask.
3. Let RB Buffer cover the dish or flask by shaking.
4. Collect cell lysate with a rubber policeman.
5. Place a Filter column in a 50 ml centrifuge tube. Apply sample lysate into the column.
6. Centrifuge at full speed for 5 minutes.
7. Discard the filter column and transfer the clarified filtrate to a new 15 ml centrifuge tube (provided by user).

RNA Binding

8. Place a RB-Maxi column in a 50 ml centrifuge tube.
9. Add half sample volume of (96-100 %) ethanol to the sample lysate from step 7 and mix immediately by vortexing. (For example, add 2.5 ml of ethanol to 5 ml of filtrate.)
10. Apply ethanol-added mixture from previous step into the RB-Maxi column.
11. Centrifuge at full speed for 5 minutes and discard the flow-through.

Optional Step: DNA residue degradation

- Add 1 ml DNase I (2 U/µl, provided by user) onto the center of the RB-Maxi column matrix. Incubate for 10 minutes at room temperature.
- Go to step 12 for Washing procedure.

Washing

12. Add 4 ml of W1 Buffer into the RB-Maxi column.
13. Centrifuge at full speed for 3 minutes.
14. Add 8 ml of Wash Buffer (ethanol added) into the RB-Maxi Column.
15. Centrifuge at full speed for 3 minutes.
16. Discard the flow-through and return the RB-Maxi Column into the 50 ml Centrifuge Tube.
17. Centrifuge again for 10 minutes at full speed to dry the column matrix.

RNA Elution

18. Place dried RB-Maxi column in a clean 50 ml centrifuge tube (RNase-free, provided by user).
19. Apply 500 µl of RNase-free water onto the center of the column matrix.
20. Stand for 5 minutes until water has been absorbed by the matrix.
21. Centrifuge at full speed for 5 minute to elute purified RNA.

Total RNA Maxi (Bacteria) Protocol

- ☐ Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required：15 ml centrifuge tube、50 ml centrifuge tube、96-100 % Ethanol、sufficient β-mercaptoethanol、DNase I (2 KU/ml, RNase-free; optional) in reaction buffer (1 M NaCl；20 mM Tris-HCl；10 mM $MnCl_2$；pH 7.0 at 25 ℃).
- ☐ For Gram-positive bacteria：Lysozyme Buffer (20 mg/ml lysozyme；20 mM Tris-HCl； 2 mM EDTA； 1% Triton X-100； pH 8.0), Prepare the lysozyme buffer just before use

Sample Preparation

For Gram-negative bacteria：

1. Transfer bacterial culture (10^9-10^{10}) into a 15 ml centrifuge tube (provided by user).
2. Centrifuge at full speed for 5 minute and discard the supernatant.
3. Add 2 ml of RT Buffer into the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 5 minutes.

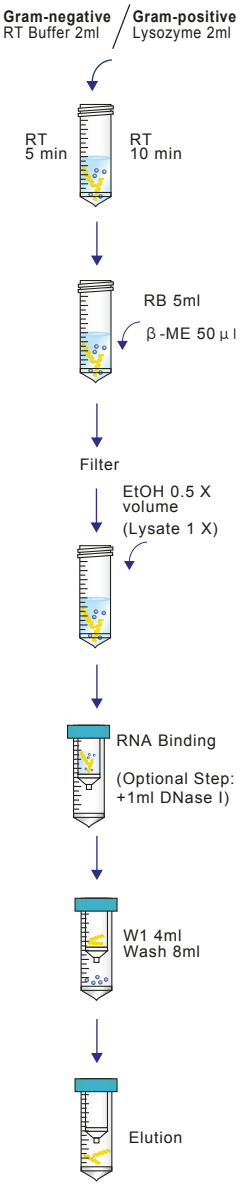
For Gram-positive bacteria：

1. Transfer bacterial culture (10^9-10^{10}) into a microcentrifuge tube (provided by user).
2. Centrifuge at full speed for 5 minute and discard the supernatant.
3. Add 2 ml 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 2-3 min.

Lysis

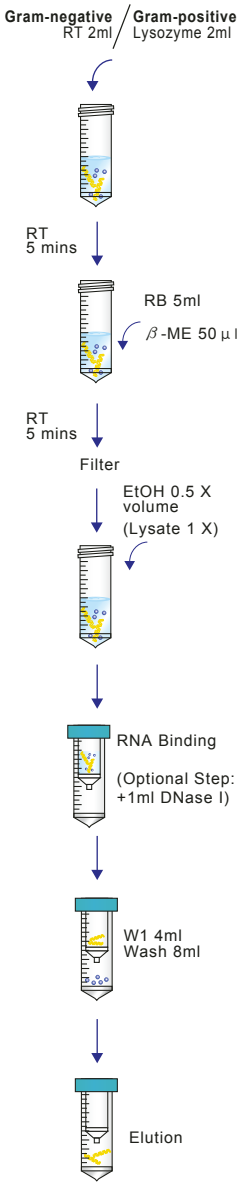
5. Add 5 ml RB of Buffer and 50 µl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing.
6. Place a Filter column in a 50 ml centrifuge tube. Apply sample mixture into the column.
7. Centrifuge at full speed for 5 minutes.
8. Discard the filter column and transfer the clarified filtrate to a new 15 ml centrifuge tube (provided by user).

ATP™ Total RNA Maxi
For Bacteria cell



ATP™ Total RNA Maxi

For Bacteria cell



RNA Binding

9. Place a RB-Maxi column in a 50 ml centrifuge tube.
10. Add a half of volume of (96-100 %) ethanol to the sample lysate from step 8 and mix immediately by vortexing. (For example, add 2.5 ml of ethanol to 5 ml of filtrate.)
11. Apply ethanol-added mixture from previous step to the RB-Maxi column.
12. Centrifuge at full speed for 5 minutes and discard the flow-through.

Optional Step: DNA residue degradation

- Add 1 ml DNase I (2 U/µl, not provided) to the center of the RB-Maxi column matrix. Stand for 10 minutes at room temperature.
- Go to step 13 for Washing procedure

Washing

13. Add 4 ml of W1 Buffer in the RB-Maxi column.
14. Centrifuge at full speed for 3 minutes.
15. Add 8 ml of Wash Buffer (ethanol added) in the RB-Maxi Column.
16. Centrifuge at full speed for 3 minutes.
17. Discard the flow-through and return the RB-Maxi Column to the 50 ml Centrifuge Tube.
18. Centrifuge again for 10 minutes at full speed to dry the column matrix.

RNA Elution

19. Place dried RB-Maxi column in a clean 50 ml centrifuge tube (RNase-free, not provided).
20. Apply 500 µl of RNase-free water onto the center of the column matrix.
21. Stand for 5 minutes until water has been absorbed by the matrix.
22. Centrifuge at full speed for 5 minute to elute purified RNA.

Total RNA Maxi (Tissue) Protocol

For best yield, Centrifuge With Swing-Bucket Rotor is recommened for following protocol.

- ☐ Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required：15 ml centrifuge tube、50 ml centrifuge tube、96-100 % Ethanol、sufficient β-mercaptoethanol、DNase I (2 KU/ml, RNase-free; optional) in reaction buffer (1 M NaCl；20mM Tris-HCl；10mM $MnCl_2$；pH 7.0 at 25 ℃).

Cell Lysis

1. Cut off 100 mg (up to 200 mg) of fresh or frozen animal tissue.
2. Grind the sample under liquid nitrogen to fine powder.
3. Add 5 ml of RB Buffer and 50 µl of β-mercaptoethanol (provided by user) to ground sample and mix by vortexing.
4. Transfer it into a 15 ml centrifuge tube (provided by user).
5. Incubate at room temperature for 5 minutes.
6. Place a Filter column in a 50 ml centrifuge tube. Apply sample mixture to the column.
7. Centrifuge for 5 minutes at full speed.
8. Discard the filter column and transfer the clarified filtrate to a new 15 ml centrifuge tube (provided by user).

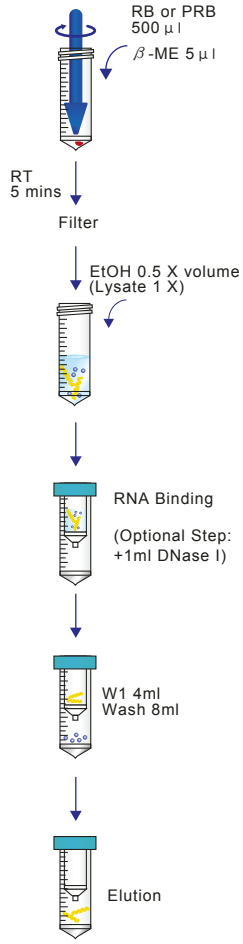
RNA Binding

9. Place a RB-Maxi column in a 50 ml centrifuge tube.
10. Add a half of volume of (96-100 %) ethanol to the sample lysate from step 8 and mix immediately by vortexing. (For example, add 2.5 ml of ethanol to 5 ml of filtrate.)
11. Apply ethanol-added mixture from previous step into the RB-Maxi column.
12. Centrifuge at full speed for 5 minutes and discard the flow-through.

Optional Step: DNA residue degradation

- Add 1 ml DNase I (2 U/µl, provided by user) onto the center of the RB column matrix. Stand for 10 minutes at room temperature.
- Go to step 13 for Washing procedure

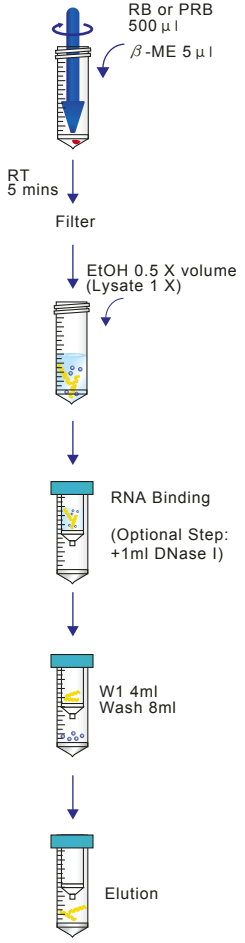
ATP™ Total RNA (Tissue)



ATP™ Total RNA Maxi Kit
(Blood/Culture cell/Bacteria/Tissue)

Store at room temperature (15~25℃)

ATP™ Total RNA (Tissue)



Washing

13. Add 4 ml of W1 Buffer in the RB-Maxi column.
14. Centrifuge at full speed for 3 minutes.
15. Add 8 ml of Wash Buffer (ethanol added) in the RB-Maxi Column.
16. Centrifuge at full speed for 3 minutes.
17. Discard the flow-through and return the RB-Maxi Column to the 50 ml Centrifuge Tube.
18. Centrifuge again for 10 minutes at full speed to dry the column matrix.

RNA Elution

19. Place dried RB-Maxi column in a clean 50 ml centrifuge tube (RNase-free, provided by user).
20. Apply 500 µl of RNase-free water onto the center of the column matrix.
21. Stand for 5 minutes until water has been absorbed by the matrix.
22. Centrifuge at full speed for 5 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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