AccuPure RNA Kits Handbook

AccuPure Cell/Blood RNA Mini Kit (96)
Cat. No. R10096

AccuPure Blood RNA X Mini Kit (96)
Cat. No. R11096

AccuPure miRNA Mini Kit (96)
Cat. No. R12096

AccuPure miRNA-900 Mini Kit (96)
Cat. No. R13096

AccuPure Tissue RNA Mini Kit (96)
Cat. No. R20096

AccuPure Plant RNA Mini Kit (96)
Cat. No. R30096

V1003.3

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Safety Precautions

Before Use INSTRUCTION

This manual is designed to assist you with the operation of following kits in advance.

AccuPure Cell/Blood RNA Mini Kit (96)

AccuPure Blood RNA X Mini Kit (96)

AccuPure miRNA Mini Kit (96)

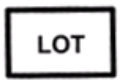
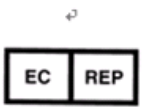
AccuPure miRNA-900 Mini Kit (96)

AccuPure Tissue RNA Mini Kit (96)

AccuPure Plant RNA Mini Kit (96)

Read it thoroughly before using the equipment or beginning any maintenance on it. These WARNINGS and CAUTIONS are intended to protect you and other persons from injuries and damages. To ensure safe operation, please follow them carefully.

Safety Symbols and Markings:

	Expiration date		Instruction for Use
	Shipment number		CAUTION! Refer to the accompanying documents.
	Production date		Recyclable Materials
	Manufacturer Information		Recyclable electrical and electronic materials.
	European Authorized Representative		CE Marking with number of the notified body.
	Temperature limit		"DO NOT REUSE"

Contents

Introduction

The *AccuPure RNA Kits Handbook* provides protocols for use with following kits:

- **AccuPure Cell/Blood RNA Mini Kit** - For purification of total RNA from whole blood, buffy coat, body fluids, lymphocytes or cultured cell.
- **AccuPure Blood RNA X Mini Kit** - For purification of total RNA from 10ml whole blood.
- **AccuPure miRNA Mini Kit** - For 200 µl plasma, serum, cell-free body fluids and cell-culture supernatants.
- **AccuPure miRNA-900 Mini Kit** - For 300 µl plasma, serum, cell-free body fluids and cell-culture supernatants.
- **AccuPure Tissue RNA Mini Kit** - For purification of total RNA from animal tissues or bacteria.
- **AccuPure Plant RNA Mini Kit** - For purification of total RNA from plant cells, plant tissues or fungi.

All AccuPure RNA Mini Kits are designed to apply on the iColumn Purification System.

Figure 1. iColumn Purification System. RNA purification using the AccuPure RNA Kits can be fully automated on the iColumn.

iColumn is a total solution for fully automated nucleic acid purification. Utilizing the silica membrane spin column method, it can purify nucleic acids with high yield and purity from wide range types of samples. In addition, through our Innovative ***Trinity Technology***™, the purification procedure can be done within a small and straight-line cartridge. Without centrifuge and vacuum pump, the workflow becomes extremely easy and different samples can be arranged in an independent channel to avoid cross contamination.

Kit Contents

AccuPure RNA Kits	Cell / Blood	Blood RNA X	miRNA	miRNA-900	Tissue RNA	Plant RNA
Cat. No.	R10096	R11096	R12096	R13096	R20096	R30096
Number of preps	96	96	96	96	96	96
Cartridge	96	96	96	96	96	96
2.0ml Sample Tube	100	-	100	100	100	100
Screw Cap	-	96	-	-	-	-
Screw Tube	-	96	-	-	-	-
2.0ml Elution Tube	100	100	100	100	100	100
1ml Tip Set	96	96	96	96	96	96
Proteinase K	-	-	4 vials	-	-	-
RL Buffer	252 ml	-	-	-	-	-
RATL Buffer	42 ml**	-	-	-	54 ml**	-
RPTL Buffer	-	-	-	-	-	54 ml**
miLP Buffer	-	-	-	28 ml	-	-
miPP Buffer	-	-	-	10 ml	-	-
RFFTL Buffer						-
DWX Buffer						-
Elution Buffer	1 vial	1 vial	1 vial	1 vial	1 vial	1 vial
Nuclease Free Water	-	-	4 vials	-	-	-

DNase I is not provided

Reagent Preparation and Storage ⚠

Protease K stock solution

*Add 1100 µl Nuclease Free Water (provided) to a Proteinase K vial to make a 10 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. Store the stock solution at -20 °C.

RATL & RPTL Buffer

**Add 1% β-Mercaptoethanel (β-ME) to RATL and RPTL Buffer freshly before use.

Carrier RNA

***Add 1350 µl Nuclease Free Water (provided) to the tube containing 1350 µl lyophilized carrier RNA to obtain a solution of 1µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently size aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.

Reagent Cartridges

Store the reagent cartridges at room temperature (15-25℃).

Intended Use

iColumn Automated DNA/RNA Purification System is intended for molecular biology application.
iColumn Automated DNA/RNA Purification System is an automated instrument for purification of nucleic acids (DNA, RNA, viral nucleic acid) from different kinds of sample by using AccuPure Kits, which develop specifically for iColumn Automated DNA/RNA Purification System. The system is intended for professional use only, but not for the diagnosis, prevention, or treatment of a disease.

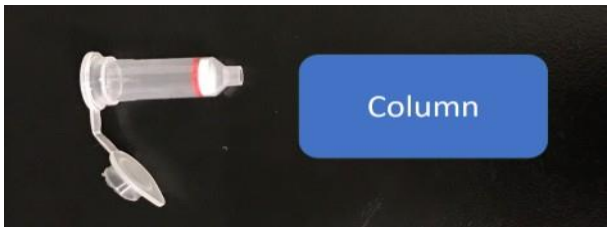
Accessories

- **Cartridge**

- **1ml Tip Sets**

- **2ml Elution/Sample Tube**

- **AccuPure Column**

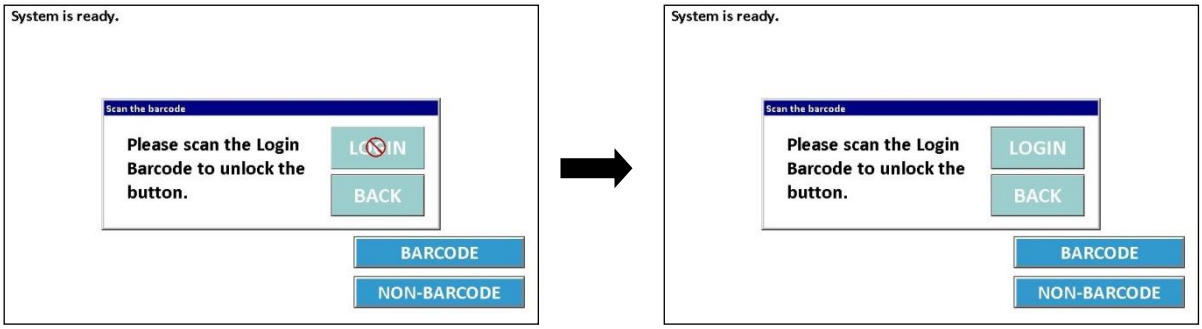


Automated RNA Purification on iColumn System

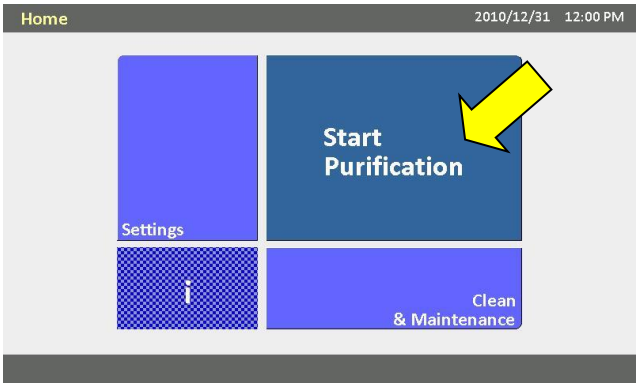
Operation Procedure- On the **Barcode** Screen

1. Turn on the iColumn System. The instrument will power up, proceed through a self-check and home all moving parts.
2. On the **Start** screen, select “BARCODE”

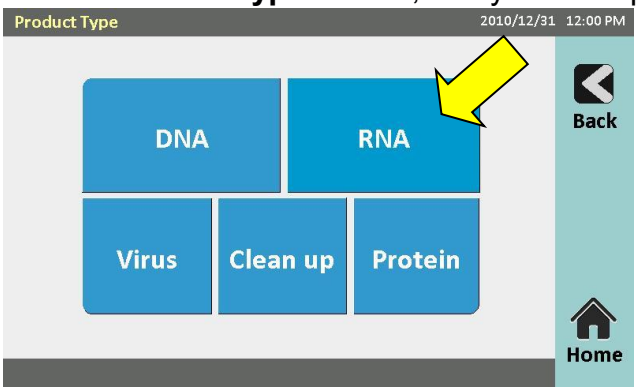
3. Scan the Login Barcode, select “Login”



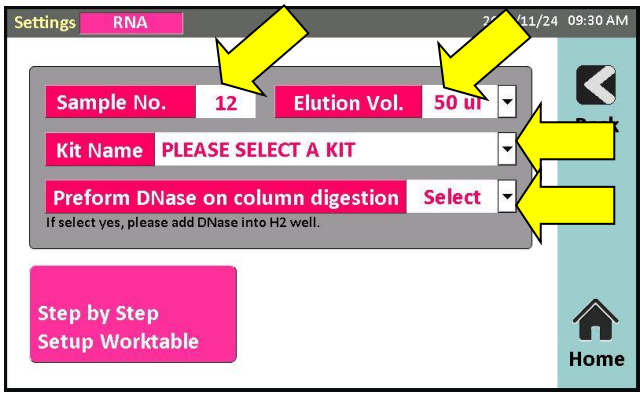
4. On the **Home** screen, select “Start Purification”.



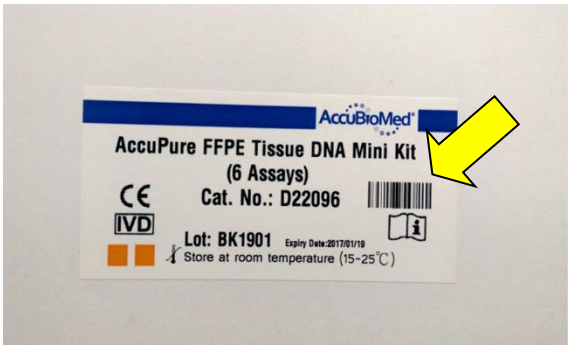
5. On the **Product Type** screen, verify the end product type.

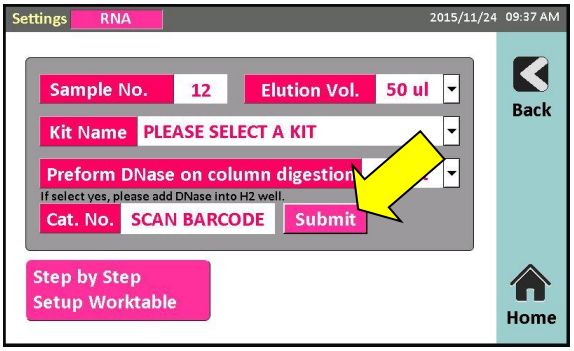


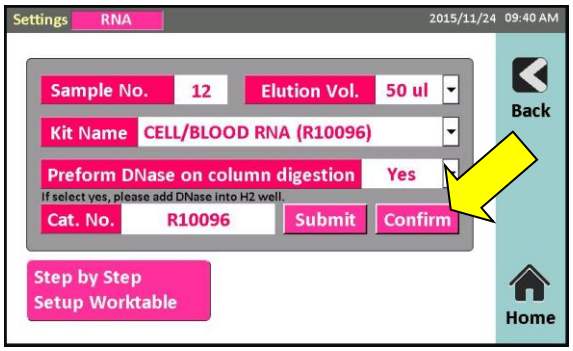
6. On the **Setting** screen
   a. Choose Sample No. - 1 to 12 preps
   b. Choose Elution Volume - 50, 100, 150 or 200 µl
   c. Choose Kit Name –
      CELL/BLOOD RNA (R10096), RNA X (R11096), miRNA (R12096), miRNA-900 (R13096), TISSUE DNA (R20096) , PLANT RNA (R30096)
   d. Choose Perform DNase on column digestion – YES or NO

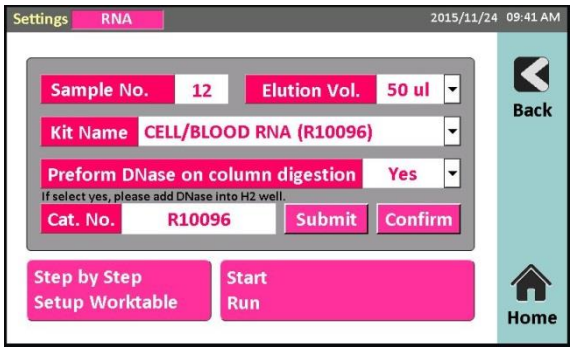


e. Scan the **Barcode** of the Cat. No. on the label of kit box and select "**Submit**" and "**Confirm**". If it matches to the Kit Name, then the "Start Run" icon pops out.

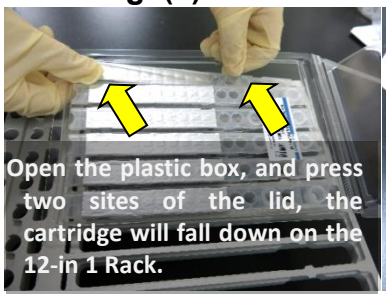


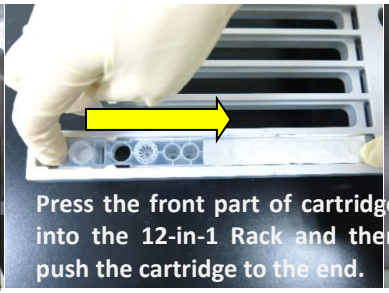


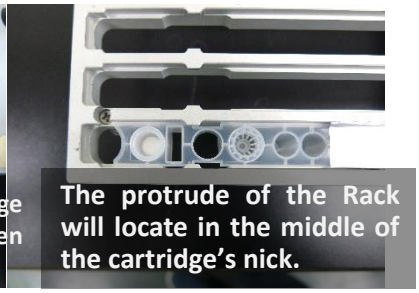




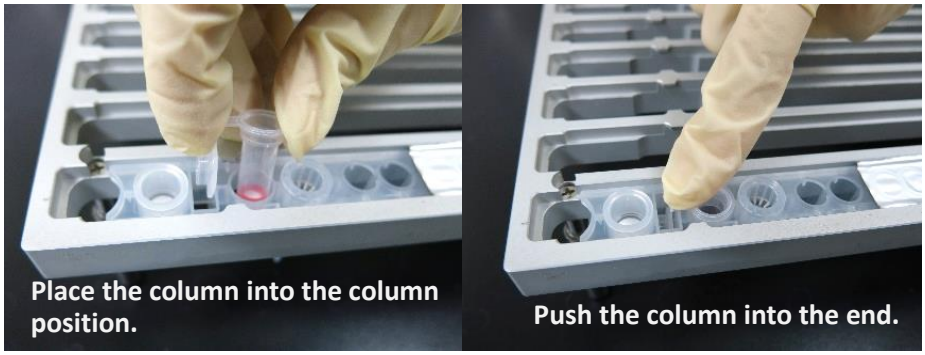
7. Open the front door and take the **12-in-1 Rack** out for preparation.
8. Load **Cartridge(s)** on the 12-in-1 Rack.



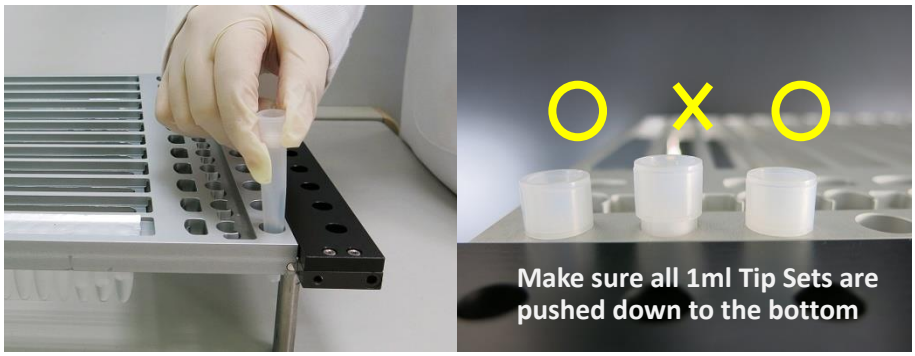




9. Place **AccuPure Column** into the column position of cartridge.

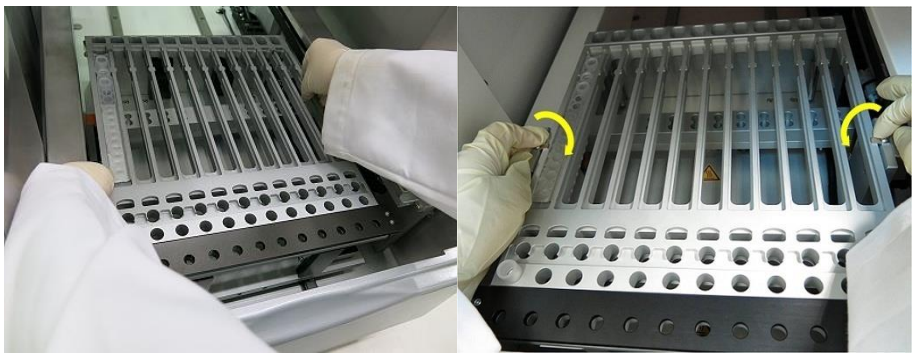


10. Load **1ml Tip Set(s)** on the 12-in-1 Rack.

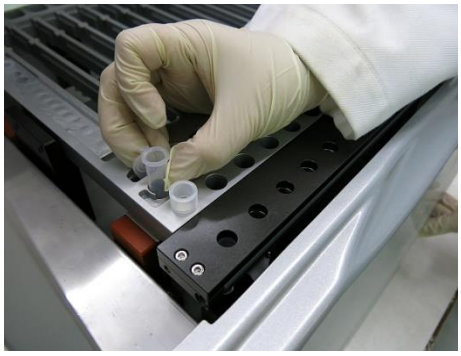


11. Load **2 ml Elution Tube(s)** on the 12-in-1 Rack and close the metal lid.

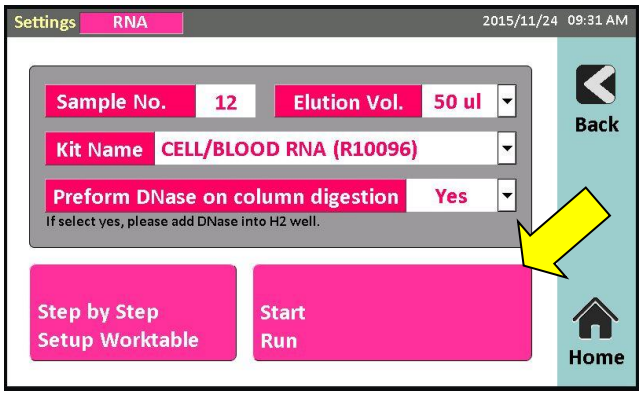
12. Place the 12-in-1 Rack into iColumn System and fix the 12-in-1 Rack by two lock plates aside the worktable.



13. Prepare samples with proper pre-treatment.
    – Please refer to **Sample Pretreatment section (Page 14).**
14. Load the **2ml Sample Tube(s)/ Screw Tube(s)** into the iColumn System.



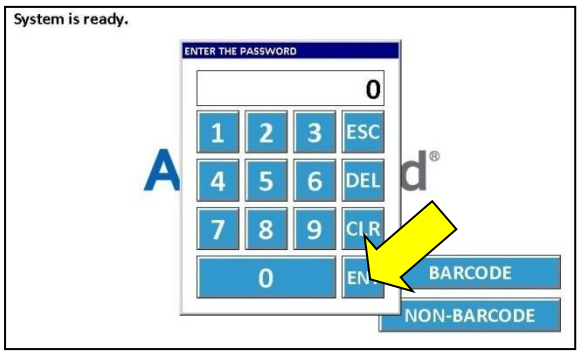
15. Close the front door.
16. Tap "**Start Run**" to start the protocol.
    (Please tap "Step by Step Setup Worktable" for guiding you how to setup the worktable step by step.)



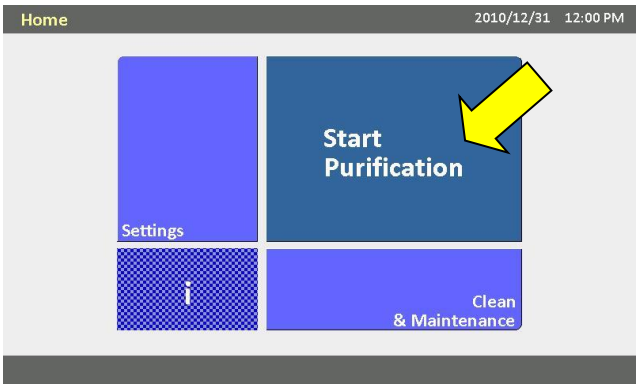
Operation Procedure- On the **Non-Barcode** Screen

1. Turn on the iColumn System. The instrument will power up, proceed through a self-check and home all moving parts.
2. On the **Start** screen, select “MON-BARCODE”

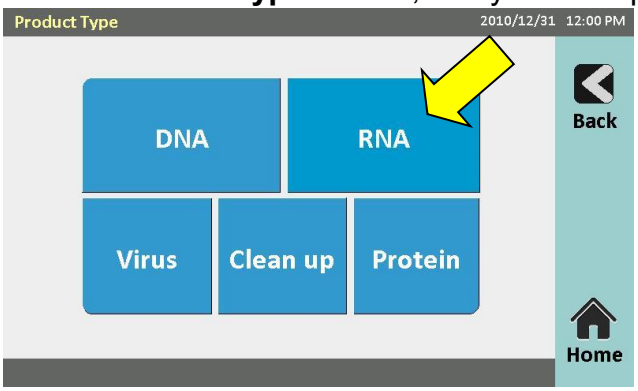
3. Enter the PASSWORD to login.



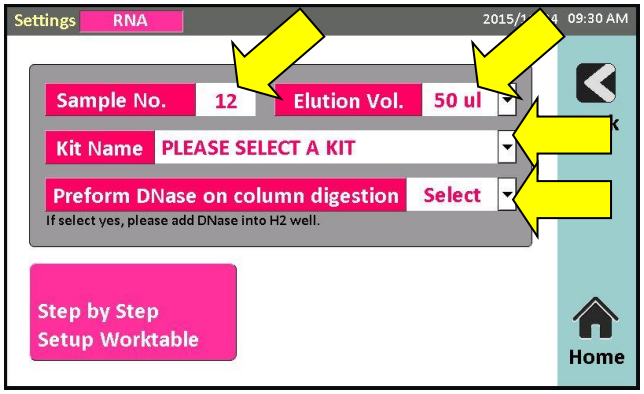
4. On the **Home** screen, select “Start Purification”.



5. On the **Product Type** screen, verify the end product type.

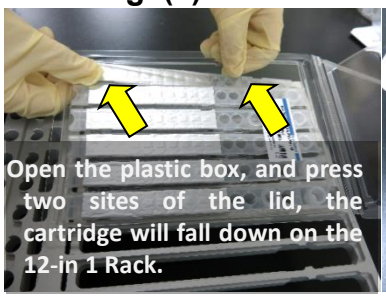


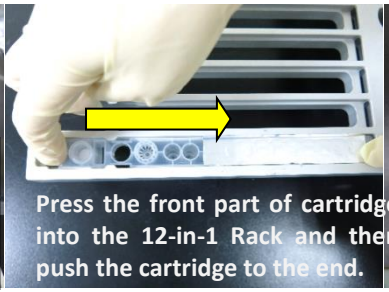
6. On the **Setting** screen
   a. Choose Sample No. - 1 to 12 preps
   b. Choose Elution Volume - 50, 100, 150 or 200 µl
   c. Choose Kit Name –
      CELL/BLOOD RNA (R10096), RNA X (R11096), miRNA (R12096), miRNA-900 (R13096), TISSUE DNA (R20096) , PLANT RNA (R30096)
   d. Choose Perform DNase on column digestion – YES or NO

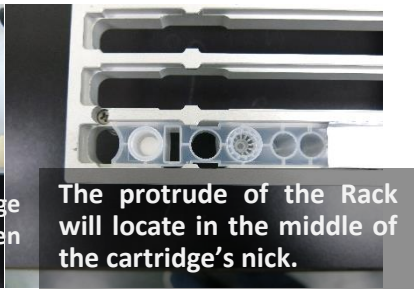


7. Open the front door and take the **12-in-1 Rack** out for preparation.

8. Load **Cartridge(s)** on the 12-in-1 Rack.

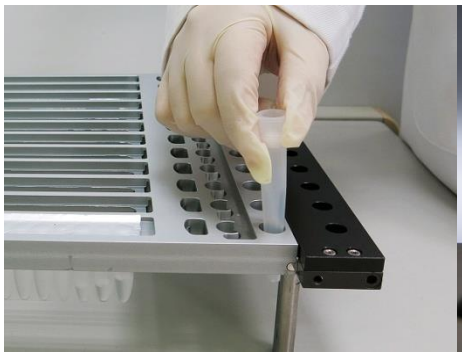






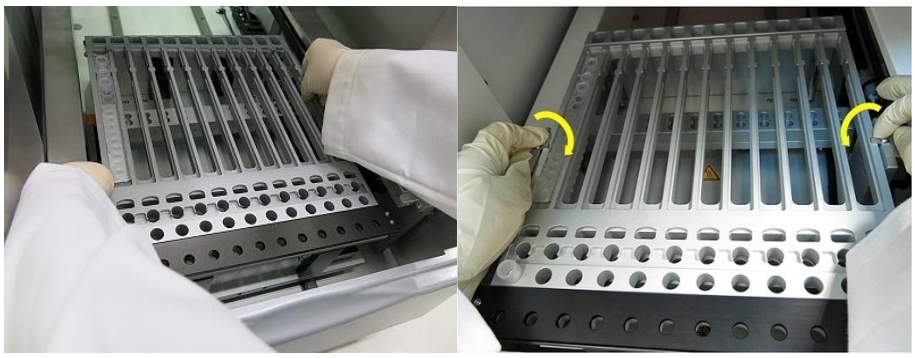
9. Place **AccuPure Column** into the column position of cartridge.

10. Load **1ml Tip Set(s)** on the 12-in-1 Rack.

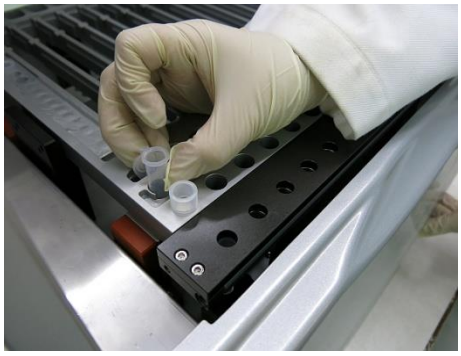


11. Load **2 ml Elution Tube(s)** on the 12-in-1 Rack and close the metal lid.

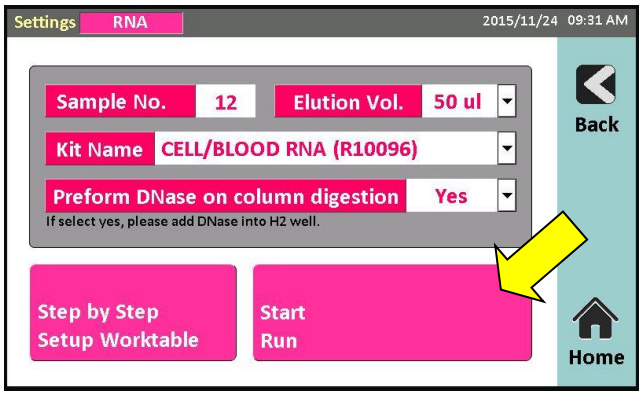
12. Place the 12-in-1 Rack into iColumn System and fix the 12-in-1 Rack by two lock plates aside the worktable.



13. Prepare samples with proper pre-treatment.
    – Please refer to **Sample Pretreatment section (Page 14).**
14. Load the **2ml Sample Tube(s)/ Screw Tube(s)** into the iColumn System.



15. Close the front door.
16. Tap "**Start Run**" to start the protocol.
    (Please tap "Step by Step Setup Worktable" for guiding you how to setup the worktable step by step.)



Sample Pretreatment

AccuPure Cell/Blood RNA Mini Kit (R10096)

I. General pretreatment for animal cells

1. Pellet 1 – 5 x 10^6 cells by centrifuge at 300 x *g* for 5 min. Remove all the supernatant.
2. Add 350 µl of RATL Buffer (add 1% β-ME freshly) to the cell pellet and vortex vigorously and brief spin down. Incubate at room temperature for 5 min.
3. (Optional) If DNA-free Total RNA is required, add RNase-free DNase I enzyme and solution (not provided) into the bottom of H2 well of Cartridge and choose “YES” on the “Perform DNase on column digestion” option on touch screen.
4. Proceed to step 14 of **Operation Procedure**.

II. For human whole blood

1. Transfer 200-300 µl whole blood to a 2 ml Sample Tube.
2. Mix 5 volumes of RL Buffer with 1 volume of the sample and mix well by inversion.
3. Incubate on ice for 10 min. Vortex briefly 2 times during incubation.
4. Centrifuge for 1 min at 4,500 rpm (1,900 x *g*) to form a cell pellet and discard the supernatant completely.
5. Add 600 µl of RL Buffer to re-suspend the cell pellet by briefly vortexing.
6. Centrifuge for 1min at 4,500 rpm (1,900 x *g*) to form a cell pellet again and discard the supernatant completely.
7. Add 350 µl of RATL Buffer (add 1% β-ME freshly) to the cell pellet and vortex vigorously. Incubate at room temperature for 5 min and brief spin down.
8. Proceed to step 14 of **Operation Procedure**.

I. For human whole blood

1. After remove erythrocyte from 10 ml human whole blood, collect leukocyte and lysis by TRIzol and BCP buffer.
2. Centrifuge the sample at 13.000 x g for 15 min at 4 °C.
3. Transfer 600 µl colorless, upper aqueous phase (containing the RNA) into Screw tube.
4. (Optional) If DNA-free Total RNA is required, add RNase-free DNase I enzyme and solution (not provided) into the bottom of H2 well of Cartridge and choose “YES” on the “Perform DNase on column digestion” option on touch screen.
5. Brief spin down, proceed to step 14 of **Operation Procedure**.

**DNase I enzyme is not provided in this kit.*

Preparation of Plasma from Blood

1. Centrifuge fresh blood sample for 10 min at 2,000 x g.
2. Remove the plasma without disturbing sedimented cells.
3. Centrifuge the separated plasma for additional 10 min at ≥ 11,000 x g.
4. Transfer supernatant for miRNA isolation.

I. For 200 µl plasma, serum, cell-free body fluids and cell-culture supernatants

1. Add 20 µl Proteinase K (20 mg/ml) into the bottom of the 2 ml Sample Tube.
2. Add 200 µl of sample to the 2 ml Sample Tube.
3. (Optional) If DNA-free Total RNA is required, add RNase-free DNase I enzyme and solution (not provided) into the bottom of H2 well of Cartridge and choose “YES” on the “Perform DNase on column digestion” option on touch screen.
4. Proceed to step 14 of **Operation Procedure**.

**DNase I enzyme is not provided in this kit.*

Preparation of Plasma from Blood

1. Centrifuge fresh blood sample for 10 min at 2,000 x g.
2. Remove the plasma without disturbing sedimented cells.
3. Centrifuge the separated plasma for additional 10 min at ≥ 11,000 x g.
4. Transfer supernatant for miRNA isolation.

I. For 900 µl plasma, serum, cell-free body fluids and cell-culture supernatants

1. Add 270 µl miLP Buffer into the bottom of the 1.5 ml micro tube (not provided).
2. Add 900 µl of sample to the 1.5 ml micro tube. Mix thoroughly by vortex 5 sec and incubate 3 min at room temperature.
3. Add 90 µl miPP Buffer, mix thoroughly by vortex 5 sec and incubate 1 min at room temperature.
4. Centrifuge for 3 min at 11,000 x g.
5. Transfer 950 µl clear supernatant to 2 ml Sample Tube.
6. (Optional) If DNA-free Total RNA is required, add RNase-free DNase I enzyme and solution (not provided) into the bottom of H2 well of Cartridge and choose “YES” on the “Perform DNase on column digestion” option on touch screen.
7. Proceed to step 14 of **Operation Procedure**.

I. General pretreatment for animal tissue

1. Weight up to 30 mg of tissue sample.
2. Grind tissue sample thoroughly with liquid nitrogen by beads beater, tissue homogenizer or mortar & pestle.
3. Add 450 µl of RATL Buffer (add 1% β-ME freshly) to the sample and mix thoroughly by vortex 30 sec. Incubate at room temperature for 5 min.
4. Centrifuge at full speed (13,000 rpm) for 2 min to spin down insoluble material and transfer 350 µl the clear supernatant to the 2 ml Sample Tube.

   *Avoid transferring any debris into the 2 ml Sample Tube. Fill up with RATL Buffer if the clear supernatant is less than 350 µl.
5. Proceed to step 14 of **Operation Procedure**.

II. For bacteria

1. Transfer 1 ml bacterial culture (up to 1 x 10^9 cells) to a micro-centrifuge tube (not provided).
2. Descend the bacterial cells by centrifuge at full speed (13,000 rpm) for 2 min and discard the supernatant completely.
3. Resuspend the cell pellet in 100 µl RNase-free lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton) (not provided).
4. Incubate at 37°C for 10 min.
5. Add 400 µl of RATL Buffer (add 1% β-ME freshly) and vortex vigorously to lyse the sample. Incubate at room temperature for 5 min.
6. Centrifuge at full speed (13,000 rpm) for 2 min to spin down insoluble material and transfer 350 µl the clear supernatant to the 2 ml Sample Tube.

   *Avoid transferring any debris into the 2ml Sample Tube. Fill up with RATL Buffer if the clear supernatant is less than 350 µl.
7. Proceed to step 14 of **Operation Procedure**.

I. General pretreatment for plant tissue (ex. Fungus, Mycelia, Maize, Rice, Tobacco, Millet)

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue.
2. Grind tissue sample thoroughly with liquid nitrogen by beads beater*, tissue homogenizer or mortar & pestle. Transfer it into a liquid nitrogen pre-chilled micro-centrifuge tube.

   *For grinding samples with beads beater. Please cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue into 2ml micro-centrifuge tube. Add proper number (1-3) and proper dimension (3-7 mm) of stainless beads into the sample tube. Immerse tube(s) into liquid nitrogen for at least 2 mins before homogenizing. Homogenize for 30 sec at 30 Hz (Do not let the tissue to thaw). Immerse sample tubes into liquid nitrogen and homogenize again, if there is still any large pieces.
3. Add 450 µl of RPTL Buffer (add 1% β-ME freshly) and vortex vigorously to lyse the sample. Incubate at room temperature for 5 min.
4. Centrifuge at full speed (13,000 rpm) for 2 min to spin down insoluble material and transfer 350 µl the clear supernatant to the 2 ml Sample Tube.

   *Avoid transferring any debris into the 2ml Sample Tube. Fill up with RATL Buffer if the clear supernatant is less than 350 µl.
5. (Optional) If DNA-free Total RNA is required, add RNase-free DNase I solution (not provided) into H2 well of Cartridge and choose “YES” on the “Perform DNase on column digestion” option on touch screen.
6. Proceed to step 14 of **Operation Procedure**.

Troubleshooting Guide⚠

Suggestions

1. Lysate cannot pass the silica membrane of spin column	
1-1. No proteinase K added in the sample pretreatment step	Stop the automatic system and repeat the RNA purification procedure with a new sample. Be sure to add proper amount of proteinase K.
1-2. Inefficient cell lysis due to decreased activity of Proteinase K	Stop the automatic system and repeat the RNA purification procedure with a new sample. Ensure that Proteinase K stock solution is store at 2-8°C.
1-3. Sample is not free from solid impurities due to improper sample pretreatment	Stop the automatic system and repeat the RNA purification procedure with a new sample. Ensure to follow sample pretreatment guide according to different samples.
2. Little RNA in the eluate	
2-1. Low concentration of cells in the sample	Input larger volume of sample (not to exceed the upper limit), and start a new round of RNA purification procedure.
2-2. Too much elution buffer	Ensure to select the proper elution volume. Larger elution volume may reduce the final RNA concentration. For samples containing less than 1μg of RNA, 50 μl of elution buffer is recommended.
2-4 Sample frozen and thawed more than once	Repeated freezing and thawing should be avoided. Always use fresh samples or samples thawed only once.
3. A260/A280 ratio for purified RNA is low	
3-1. Sample is not fresh due to too long maintenance	Use fresh or properly stored sample and Repeat the RNA purification procedure.

3-2. Inefficient cell lysis due to decreased activity of Proteinase K	Repeat the RNA purification procedure with a new sample. Ensure that Proteinase K stock solution is store at 2-8°C.
4. DNA contamination	
4-1. DNA present in the sample	To avoid copurification of DNA, use of cell-free body fluids for preparation of viral RNA is recommended. Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, and most swabs, should be made cell-free by centrifuge, pellet the cells for 10 min at 1500 x g and use supernatant for isolation of viral RNA. If DNA-free RNA is required, digest either the sample or the eluate with RNase-free DNase. DNase in the eluate must be inactivated by heat treatment (15 min, 70 °C).
5. RNA degraded	
5-1. Harvested tissue not immediately stabilized	Submerge the tissue in the appropriate volume.
5-2. Too much tissue for proper stabilization	Reduce the amount of tissue.
5-3. Tissue too thick for stabilization	Cut large samples into slices less than 0.5 cm.
5-4. Inappropriate handling of starting material	For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at -70°C.

Ordering Information

Product Type	Product Name	Cat. No.
System	iColumn 12 Automated DNA/RNA Purification System	ABM1012
	iColumn 24 Automated DNA/RNA Purification System	ABM1024
	iColumn LV8 Automated DNA/RNA Purification System	ABM2008
DNA	AccuPure Cell/Blood DNA Mini Kit (96)	D10096
	AccuPure Circulating DNA Mini Kit (96)	D11096
	AccuPure Tissue DNA Mini Kit (96)	D20096
	AccuPure FFPE Tissue DNA Mini Kit (96)	D22096
	AccuPure MTB DNA Mini Kit (96)	D23096
	AccuPure Stool DNA Mini Kit (96)	D24096
	AccuPure Plant DNA Mini Kit (96)	D30096
RNA	AccuPure Cell/Blood RNA Mini Kit (96)	R10096
	AccuPure Blood RNA X Mini Kit (96)	R11096
	AccuPure miRNA Mini Kit (96)	R12096
	AccuPure miRNA-900 Mini Kit (96)	R13096
	AccuPure Tissue RNA Mini Kit (96)	R20096
	AccuPure Plant RNA Mini Kit (96)	R30096
Virus	AccuPure Viral DNA /RNA Mini Kit (96)	T10096
	AccuPure HPV DNA Mini Kit (96)	T12096
LV DNA	AccuPure Circulating DNA Mini Kit-LV3 (96)	D11096-LV3
	AccuPure Circulating DNA Mini Kit-LV5 (96)	D11096-LV5

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