

AccuPure Viral Kits

Handbook

AccuPure Viral DNA/RNA Mini Kit (96)

Cat. No. T10096

AccuPure HPV DNA Mini Kit (96)

Cat. No. T12096

V1005.0



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Safety Precautions

Before Use INSTRUCTION

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



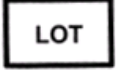





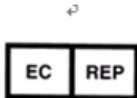



AccuPure Viral DNA/RNA Mini Kit (96)

AccuPure HPV DNA 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"



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Introduction

The *AccuPure Viral Kits Handbook* provides protocol for use with following kit:

- **AccuPure Viral DNA/RNA Mini Kit** - For purification of viral DNA/RNA from plasma, serum, cell-free body fluids and cell-culture supernatants.
- **AccuPure HPV DNA Mini Kit** - For purification of Human Papillomavirus from cytology brush in preserving tube and pelleted cells.

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



Figure 1. iColumn Purification System. Viral DNA/RNA purification using the AccuPure Viral 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.

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.



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Kit Contents

AccuPure DNA Kits	Viral	HPV DNA
	DNA/RNA	Mini Kit
Cat. No.	T10096	T12096
Number of preps	96	96
Cartridge	96	96
2.0 ml Sample Tube	100	100
2.0 ml Elution Tube	100	100
1ml Tip Set	96	96
AccuPure G Column	—	96
AccuPure R Column	96	—
Proteinase K	2 vial*	2 vial*
Carrier RNA	0.5 vial**	0.5 vial**
Elution Buffer	1 vial	1vial
Nuclease Free Water	3 vial	3 vial

Reagent Preparation and Storage

Protease K stock solution

*Add 1100 µl Nuclease Free Water to the 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.

Carrier RNA

**Add 1350 µl Nuclease Free Water to the Carrier RNA vial. Vortex and make sure that Carrier RNA has been completely dissolved, then dividing it into aliquots and store it at -20°C. Do not freeze thaw the aliquots of carrier RNA more than 3 times.

When purchase two boxes of kits which contain 0.5 vial of Carrier RNA, one Carrier RNA vial will be provided in one of two boxes and showed on the label of box.

Reagent Cartridges

Store the reagent cartridges dry at room temperature (15-25°C)



Accessories

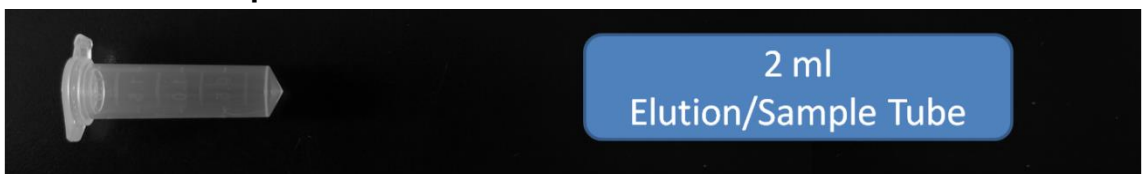
- Cartridge



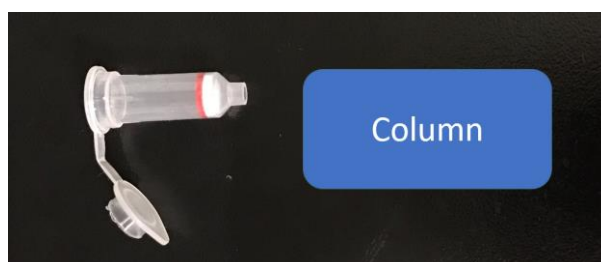
- 1 ml Tip Sets



- 2 ml Elution/Sample Tube



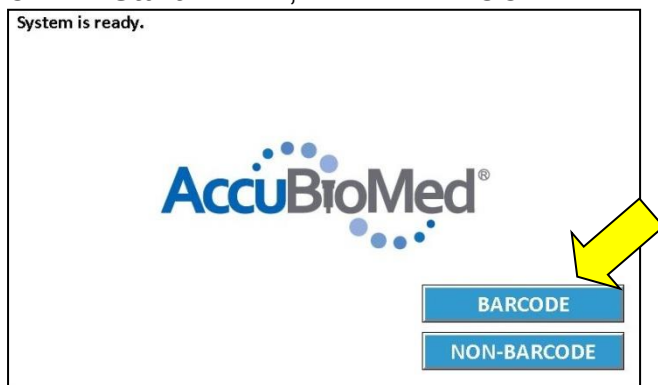
- AccuPure Column



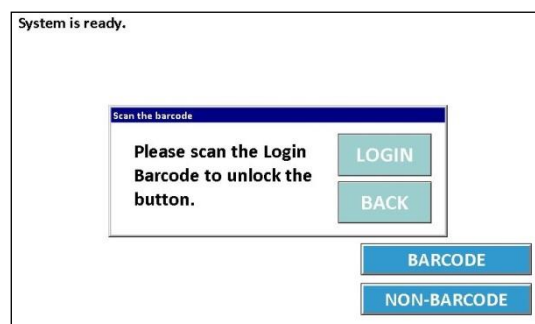
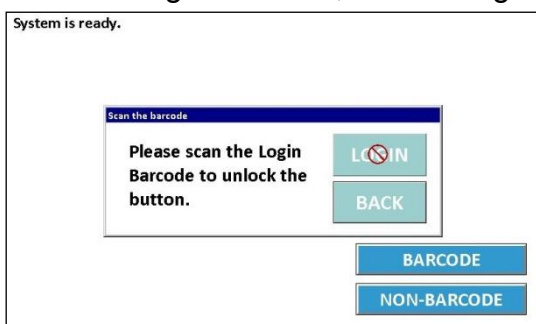
Automated RNA Purification on iColumn System

Operation Procedure- On the **Barcode** Screen

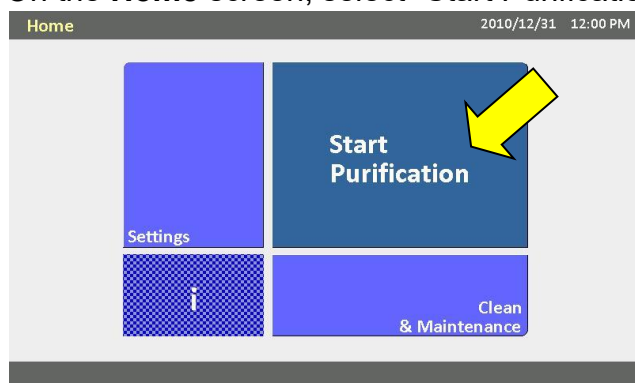
1. Turn on the iColumn System. The instrument will power up, proceed through a self-check and home all moving parts.
2. On the **Start** screen, select "BARCODE"



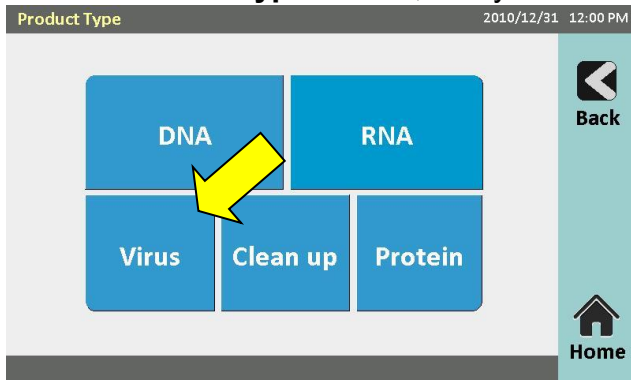
3. Scan the Login Barcode, select "Login"



4. On the **Home** screen, select "Start Purification".

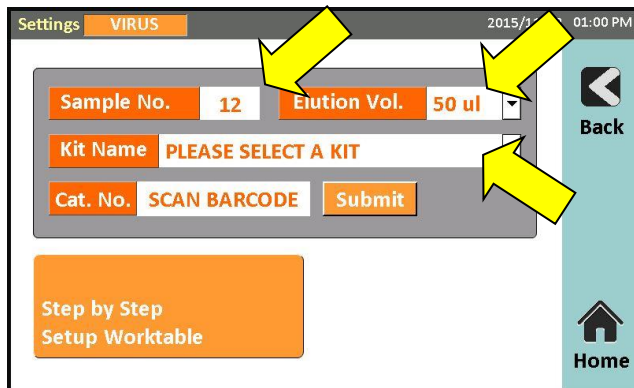


5. On the **Product Type** screen, verify the end product type.

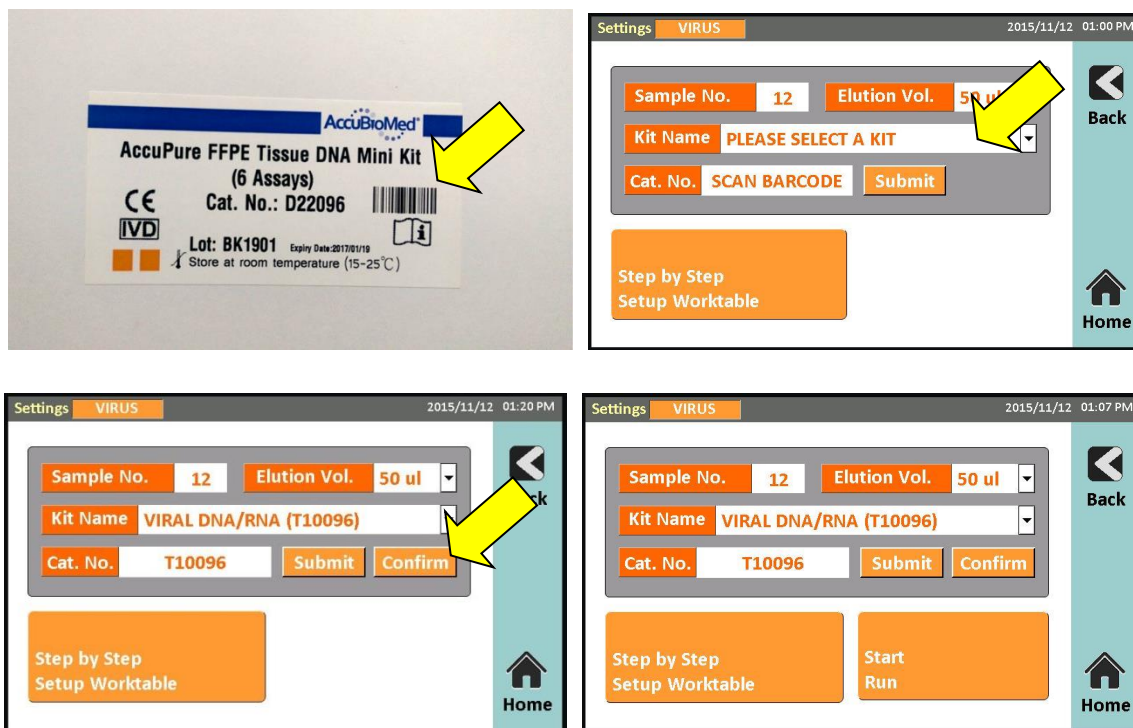


6. On the **Setting** screen

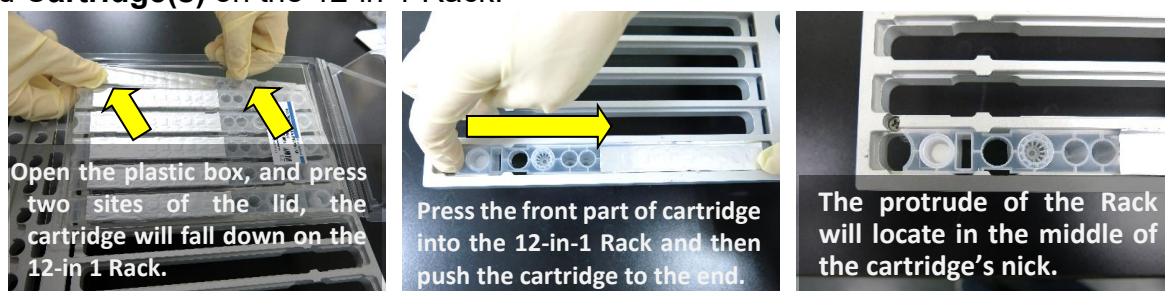
- a. Choose Sample No. - 1 to 12 preps for iColumn 12; 1 to 24 for iColumn 24
- b. Choose Elution Volume – 50 µl
- c. Choose Kit Name – VIRAL DNA/RNA (T11096)



- d. 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.



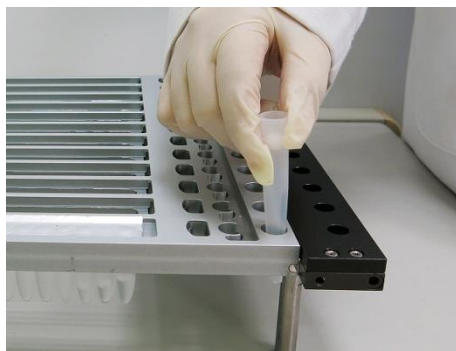
7. Open the front door and take the **12-in-1 Rack** out for preparation.
(Please tap “Step by Step Setup Worktable” for guiding you how to setup the worktable step by step.)
8. Load **Cartridge(s)** on the 12-in-1 Rack.



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



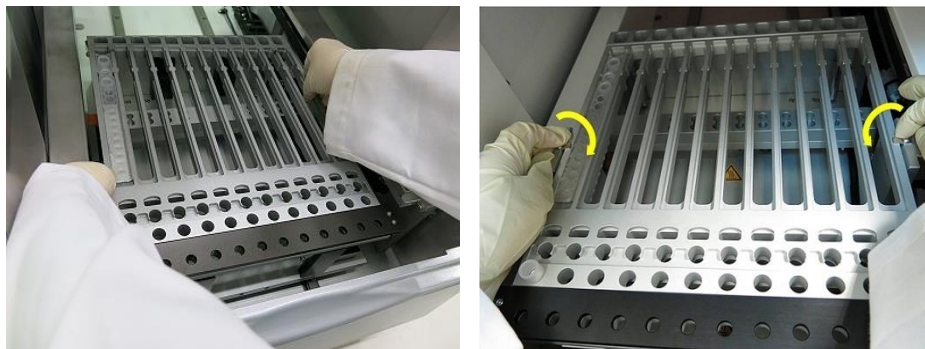
10. Load **1 ml Tip Set(s)** on the 12-in-1 Rack



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



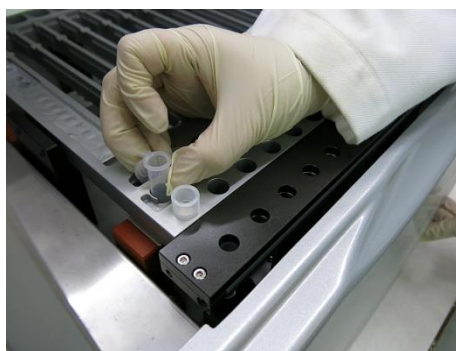
12. Place the 12-in-1 Rack into iColumn System and fix the 12-in-1 Rack by two lock plate aside the worktable.



13. Prepare samples with proper pre-treatment.

– Please refer to **Sample Pretreatment section (Page 16)**.

14. Load the **2 ml Sample Tube(s)** into the iColumn System.



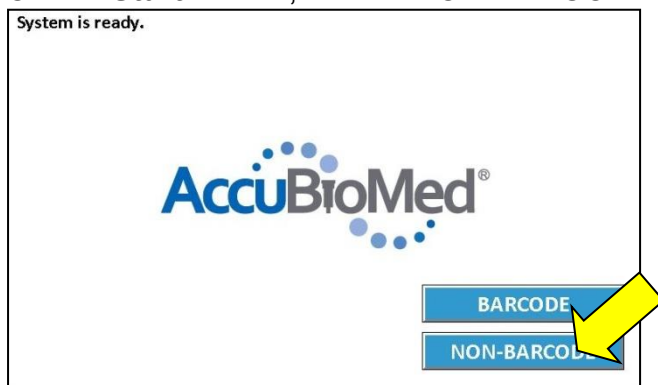
15. Close the front door.

16. Tap **“Start Run”** to start the protocol.

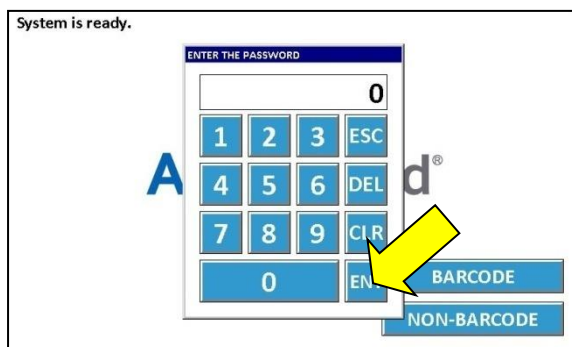


Operation Procedure- On the **Non-Barcode** Screen

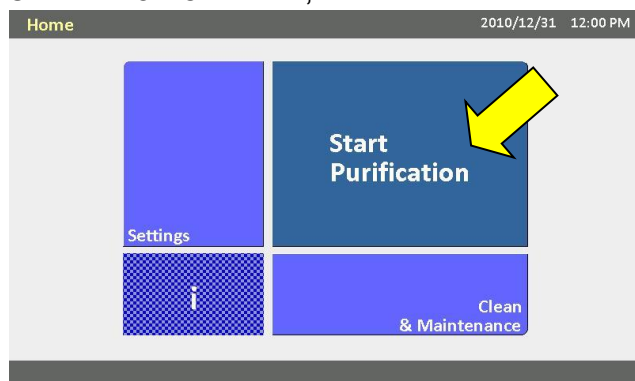
1. Turn on the iColumn System. The instrument will power up, proceed through a self-check and home all moving parts.
2. On the **Start** screen, select "MON-BARCODE"



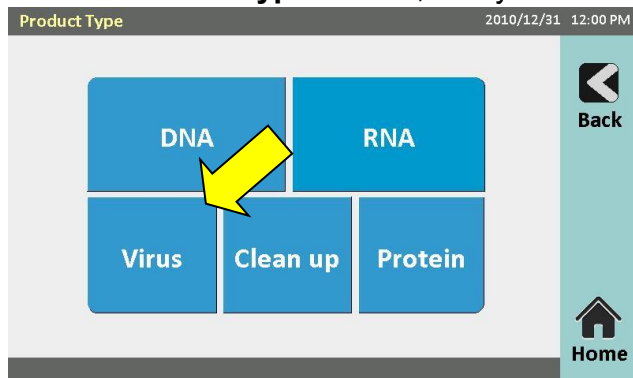
3. Enter the PASSWORD to login.



4. On the **Home** screen, select "Start Purification".



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



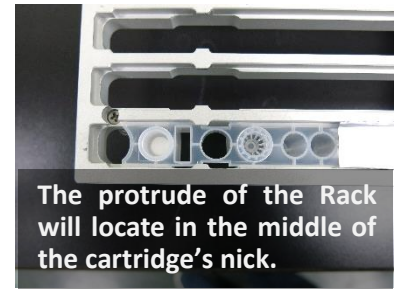
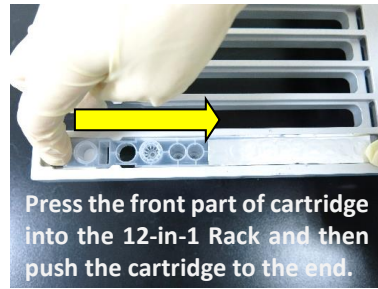
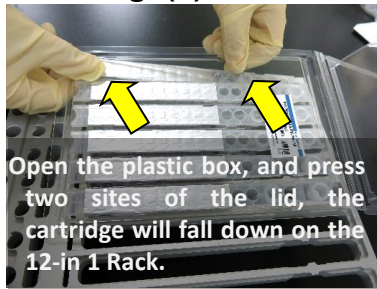
6. On the **Setting** screen
- Choose Sample No. - 1 to 12 preps for iColumn 12; 1 to 24 for iColumn 24
 - Choose Elution Volume – 50 μ l
 - Choose Kit Name – VIRAL DNA/RNA (T11096)
 - Choose Mix Internal Control – “YES” or “No”
(If choose “YES”, please add internal control at the bottom of H2 well. The 230 μ l lysis buffer will mix at H2 well, and 200 μ l mixed lysis buffer will add to sample.)



7. Open the front door and take the **12-in-1 Rack** out for preparation.
(Please tap “Step by Step Setup Worktable” for guiding you how to setup the worktable step by step.)



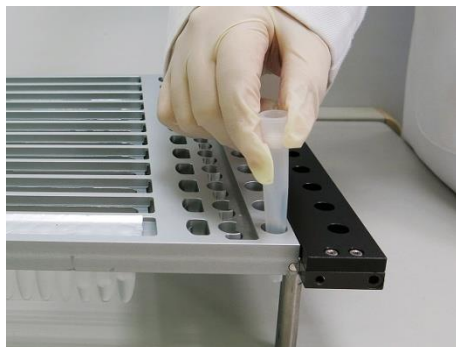
8. Load **Cartridge(s)** on the 12-in-1 Rack.



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



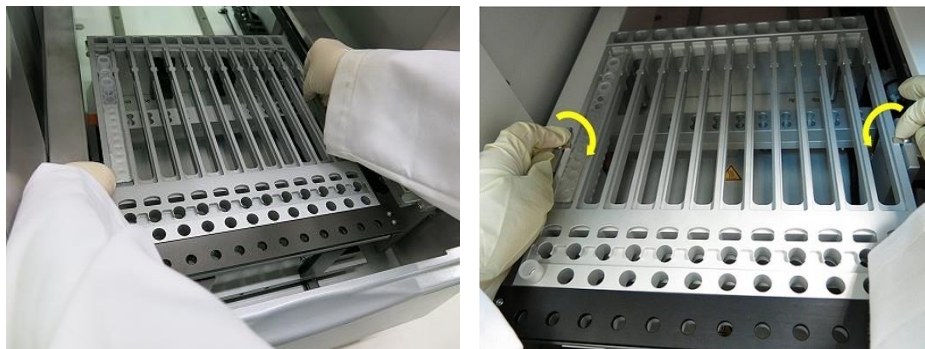
10. Load **1 ml Tip Set(s)** on the 12-in-1 Rack



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



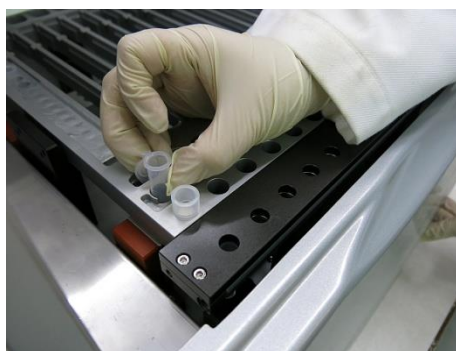
12. Place the 12-in-1 Rack into iColumn System and fix the 12-in-1 Rack by two lock plate aside the worktable.



13. Prepare samples with proper pre-treatment.

– Please refer to **Sample Pretreatment section (Page 16)**.

14. Load the **2 ml Sample Tube(s)** into the iColumn System.



15. Close the front door.

16. Tap “**Start Run**” to start the protocol.



Sample Pretreatment

AccuPure Viral DNA/RNA Mini Kit (T10096)

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

1. Add 20 µl Proteinase K into the bottom of the 2 ml Sample Tube.
2. Add 6 µl Carrier RNA into the 2 ml Sample Tube.
3. Add 200 µl of sample to the 2 ml Sample Tube.
4. Proceed to step 14 of **Operation Procedure**.

II. For animal tissue

1. Weight up to 25 mg of animal tissue or no more than 10 mg spleen tissue.
 2. Homogenize tissue samples by one of following methods.
 - A. Homogenize tissue sample with liquid nitrogen.
Grind tissue sample thoroughly with liquid nitrogen by beads beater, tissue homogenizer or mortar & pestle. Proceed with step 3.
 - B. Homogenize tissue sample with buffer.
Place tissue sample into 2 ml micro-centrifuge tube containing 150 µl PBS. Homogenize samples with homogenizer thoroughly. Add 150 µl TATL Buffer (1% β-ME added) and vortex for 30s, then proceed to step 4.
 3. Add 300 µl TATL* Buffer (1% β-ME added) and mix thoroughly by vortex 30s.
 4. Add 20 µl Proteinase K, mix by vortex for 30s.
 5. Incubate at room temperature for 5 min or until the tissue is completely lysed (vortex occasionally during incubation).
 6. Centrifuge at 13,000 rpm for 5 minutes and transfer clear lysate to the 2 ml Sample Tube. (avoid to aspirate any debris)
 7. Add 6 µl Carrier RNA into the 2 ml Sample Tube.
 8. Proceed to step 14 of **Operation Procedure**.
- *TATL buffer should be bought separately.



AccuPure HPV DNA Mini Kit (T12096)

I. For cytology brush in preserving tube

1. Check the media volume of preserving tube, add equal volume of TATL buffer into preserving tube then mix by vigorously vortex for 30s.
2. Incubate 30min at 60°C heat bath.
3. Add 20 µl Proteinase K into the bottom of the 2 ml Sample Tube.
4. Add 6 µl Carrier RNA into the 2 ml Sample Tube.
5. Add 200 µl lysed sample to 2 ml Sample Tube. (Avoid aspirating any mucus or cell debris.)
6. Proceed to step 14 of **Operation Procedure**.

II. For pelleted cells

1. Pellet cells from preserving media by centrifuge at 300 x g for 5 min in micro-centrifuge tube (not provided). Remove all the supernatant.
2. Add 200 µl TATL buffer to cell pellet and vortex vigorously.
3. Add 20 µl Proteinase K into the micro-centrifuge tube.
4. Incubation at 60°C for 15 min.
5. Centrifuge at 13,000 rpm for 3 min.
6. Transfer 200 µl supernatant to the 2 ml Sample Tube.
7. Add 6 µl Carrier RNA into the 2 ml Sample Tube.
8. Proceed to step 14 of **Operation Procedure**.

III. For LBC (Liquid-Based cytology)

1. Add 80 µl TATL Buffer to 2 ml Sample Tube.
2. Transfer 250 µl liquid media to 2 ml Sample Tube. Vortex vigorously and brief spin down.
3. Add 20 µl Proteinase K into 2 ml Sample Tube. Mix by vortex.
4. Add 6 µl Carrier RNA into 2 ml Sample Tube. Mix by vortex and brief spin down.
5. 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 DNA concentration. For samples containing less than 1µg of RNA, 50 µl of elution buffer is recommended.
2-3 Degraded carrier RNA	Carrier RNA was not stored at -20°C or underwent multiple freeze-thaw cycles.
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.
2-5 RNA degraded	Often RNA is degraded by RNase in the starting material (plasma, serum, body fluids). Ensure that the samples are processed quickly. If necessary, add



	RNase inhibitor to the sample. Check for RNase contamination of buffers and water, and ensure that no RNase is introduced during the procedure.
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 co purification 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. General handling	
5-1. Clogged membrane	Cryoprecipitate have formed in plasma due to repeated freezing and thawing. Do not use plasma that has been frozen and thawed more than once.



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



Contact



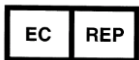
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