AccuPure DNA Kits Handbook

AccuPure Cell/Blood DNA Mini Kit (96)
Cat. No. D10096

AccuPure Circulating DNA Mini Kit (96)
Cat. No. D11096

AccuPure Tissue DNA Mini Kit (96)
Cat. No. D20096

AccuPure FFPE Tissue DNA Mini Kit (96)
Cat. No. D22096

AccuPure MTB DNA Mini Kit (96)
Cat. No. D23096

AccuPure Stool DNA Mini Kit (96)
Cat. No. D24096

AccuPure Plant DNA Mini Kit (96)
Cat. No. D30096

V1003.1

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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 DNA Mini Kit (96)

AccuPure Circulating DNA Mini Kit (96)

AccuPure Tissue DNA Mini Kit (96)

AccuPure FFPE Tissue DNA Mini Kit (96)

AccuPure MTB DNA Mini Kit (96)

AccuPure Stool DNA Mini Kit (96)

AccuPure Plant 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
LOT	Shipment number		CAUTION! Refer to the accompanying documents.
	Production date		Recyclable Materials
	Manufacturer Information		Recyclable electrical and electronic materials.
EC REP	European Authorized Representative	CE	CE Marking with number of the notified body.
	Temperature limit		"DO NOT REUSE"

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CE IVD

Content

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Introduction

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

- **AccuPure Cell/Blood DNA Mini Kit** - For purification of total DNA from whole blood, buffy coat, body fluids, lymphocytes or cultured cells.
- **AccuPure Circulating DNA Mini Kit** - For purification of cell free DNA from plasma or body fluids.
- **AccuPure Tissue DNA Mini Kit** - For purification of total DNA from animal tissues, or bacteria.
- **AccuPure FFPE Tissue DNA Mini Kit** - For purification of total DNA from FFPE tissue.
- **AccuPure MTB Tissue DNA Mini Kit** - For purification of total DNA from MTB.
- **AccuPure Stool Tissue DNA Mini Kit** - For purification of total DNA from stool.
- **AccuPure Plant DNA Mini Kit** - For purification of total DNA from plant cells, plant tissues or fungi.

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

Figure 1. iColumn Purification System. DNA purification using the AccuPure DNA 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.

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Kit Contents

AccuPure DNA Kits	Cell / Blood	Circulating	Tissue	FFPE	MTB	Stool	Plant
Cat. No.	D10096	D11096	D20096	D22096	D23096	D24096	D30096
Number of preps	96	96	96	96	96	96	96
Cartridge	96	96	96	96	96	96	96
2.0ml Sample Tube	100	-	100	100	-	100	100
Screw Cap	-	96	-	-	96	-	-
Screw Tube	-	96	-	-	96	-	-
Beads Tube	-	-	-	-	-	96	-
2.0ml Elution Tube	100	100	100	100	100	100	100
1ml Tip Set	96	96	96	96	96	96	96
AccuPure R Column	–	–	–	96	–	–	–
AccuPure G Column	96	96	96	–	96	96	96
Proteinase K	2 vial*	8 vial**	2 vial*	-	2 vial*	2 vial*	-
PK Plus				3 vial#			
Carrier RNA	-	1 vial***	-	-	-	-	-
DL Buffer	-	96 ml	-	-	-	-	-
DATL Buffer	-	-	24 ml	36 ml	24 ml	-	-
DPTL1-S Buffer	-	-	-	-	-	-	48 ml
DPTL2-S Buffer	-	-	-	-	-	-	16 ml
DWX Buffer	-	-	-	60 ml	-	-	-
DSTL Buffer	-	-	-	-	-	120 ml	-
Elution Buffer	1 vial	1 vial	1 vial	1 vial	1 vial	1 vial	1 vial
Nuclease Free Water	2 vial	4 vial	2 vial	-	2 vial	2 vial	-
PK Plus Solution	-	-	-	3 vial	-	-	-

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Reagent Preparation and Storage ⚠

Proteinase K stock solution for D10096. D20096. D23096. D24096

*Add 1100 µl Nuclease-free Water to 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℃ (1 year) or 4℃ (3 months)**.

Proteinase K stock solution for D11096

Add 550 µl Nuclease-free Water to Proteinase K vial to make a **20 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at -20℃ (1 year) or 4℃ (3 months)**.

Carrier RNA

***Add 1350 µl Nuclease free Water to the tube containing 1350 µg lyophilized carrier RNA to obtain a solution of **1µg/µl**. Dissolve the carrier RNA thoroughly, divide it into conveniently size aliquots (50-100 µl/ tube), and **store it at -20℃**. **Do not freeze-thaw the aliquots of carrier RNA more than 3 times**.

PK Plus stock solution for D22096

#Add 1100 µl PK Plus Solution to PK Plus vial to make a **20 mg/ml** stock solution. Vortex and make sure that PK Plus has been completely dissolved. **Store the stock solution at -20℃ (1 year) or 4℃ (3 months)**.

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.

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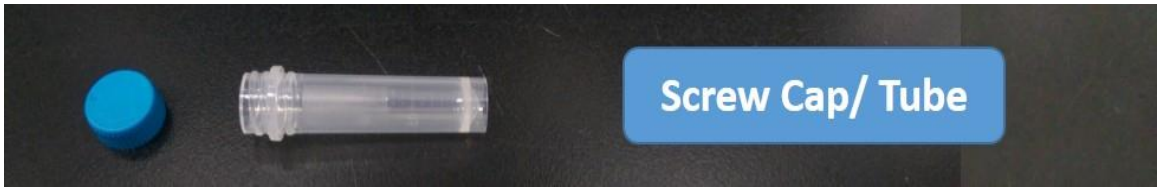
Accessories

- **Cartridge**

- **1ml Tip Sets**

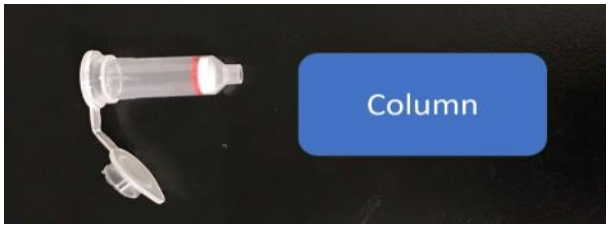
- **2ml Elution/Sample Tube**

- **Screw Cap/Tube(For D11096. D23096)**



- **Beads Tube (For D24096)**

- **AccuPure Column**



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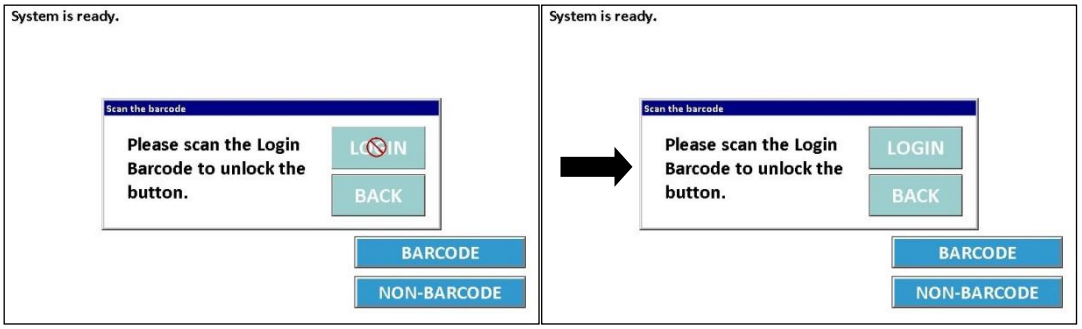
CE IVD

Automated DNA Purification on iColumn System

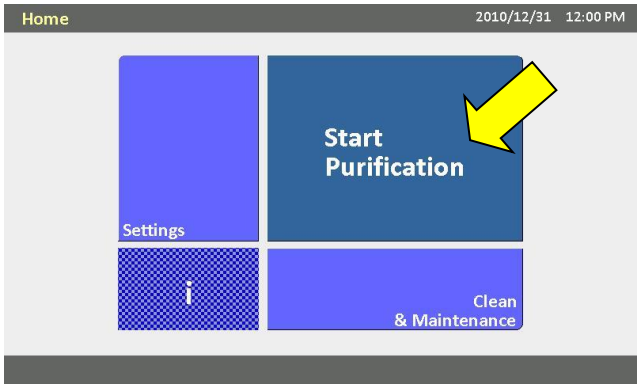
Operation Procedure- On the **Barcode** Screen

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**”.



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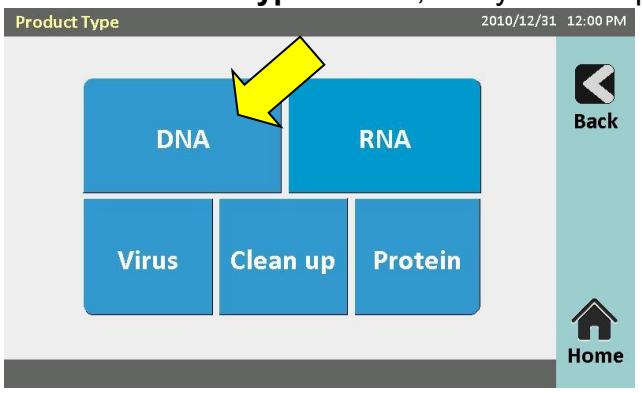
5. On the **Product Type** screen, verify the end product type.



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 Vol.** - 50, 100, 150 or 200 µl
   c. Choose **Kit Name**-
   CELL/BLOOD DNA (D10096), CIRCULATING DNA(D11096), TISSUE DNA (D20096), FFPE TISSUE DNA (D22096),MTB DNA (D23096),STOOL DNA (D24096), PLANTDNA (D30096).

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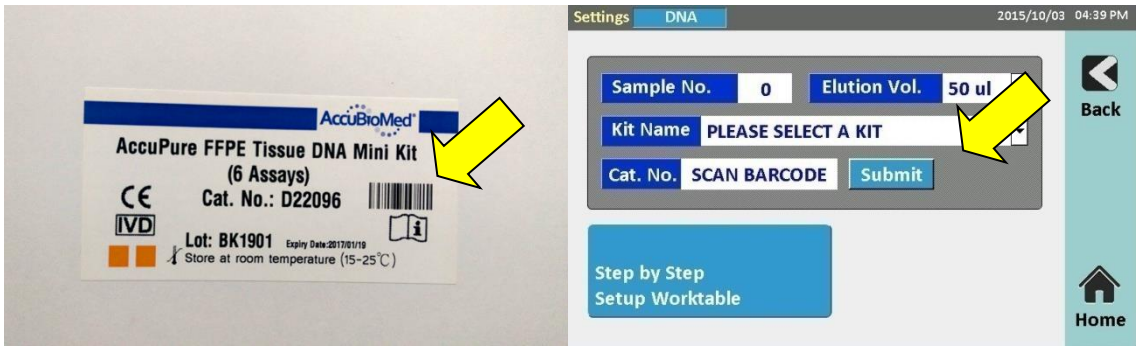
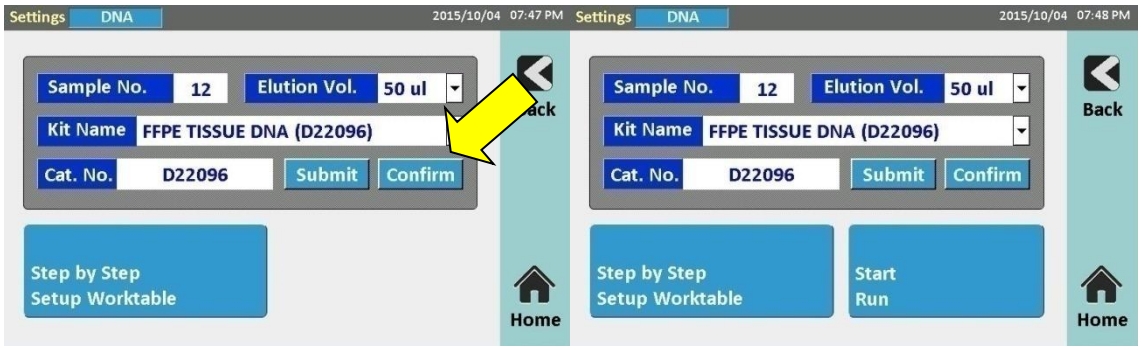
d. Scan the **Barcode** of the **Cat. No.** on the label of kit box and select "**Submit**" and "**Confirm**". If it matches to the Kit Name, then the "**Start Run**" icon pops out.





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.)

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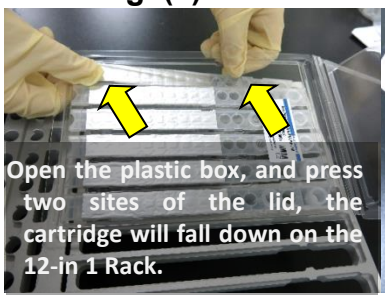
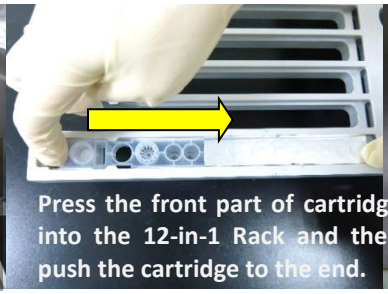
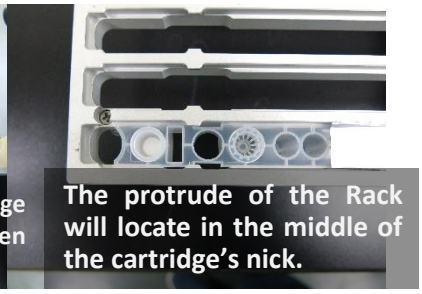
8. Load **Cartridge(s)** on the 12-in-1 Rack.

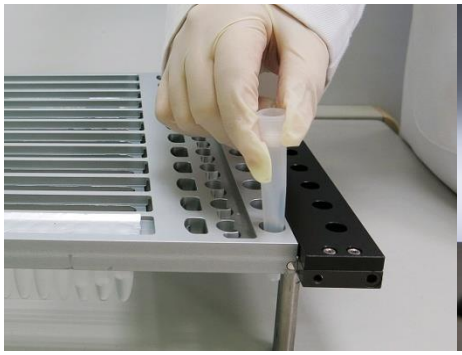






9. Place **AccuPure Column** into the column position of cartridge.

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.

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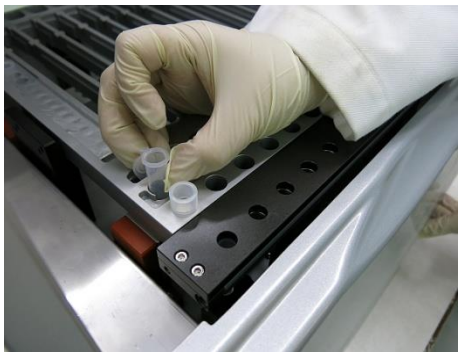
12. Place the 12-in-1 Rack into iColumn System and fix the 12-in-1 Rack by two lock plates aside the worktable.

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.

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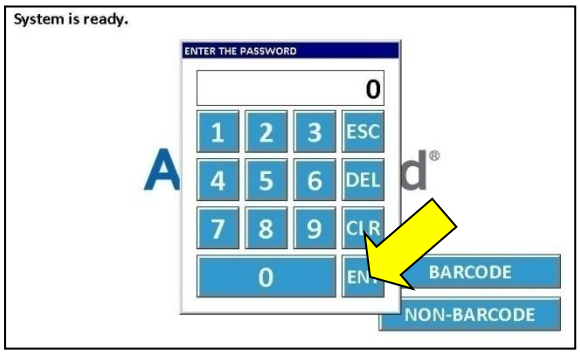
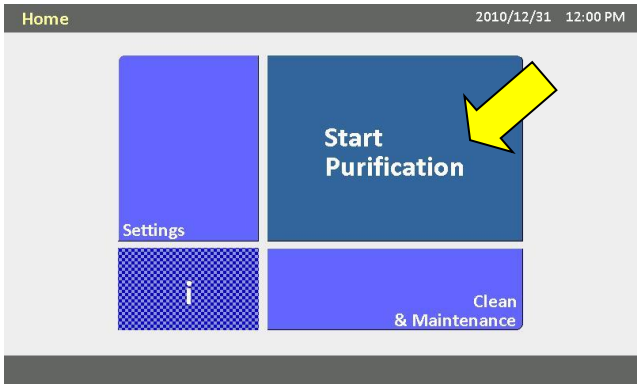
Operation Procedure- On the **Non-Barcode** Screen

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, and select "**MON-BARCODE**"

3. Enter the **PASSWORD** to login.



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



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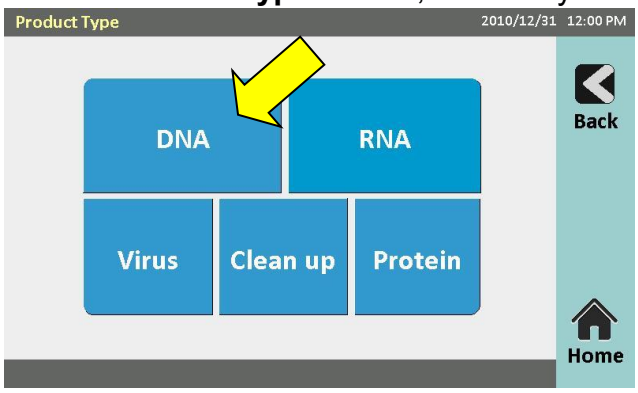
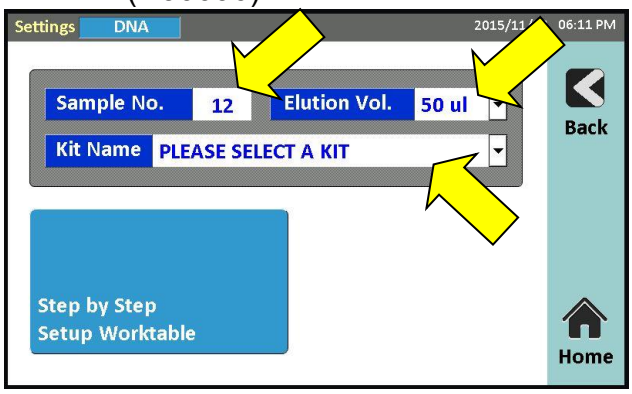
5. On the **Product Type** screen, and verify the end product type.



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 Vol**. - 50, 100, 150 or 200 µl
   c. Choose **Kit Name**-
   CELL/BLOOD DNA (D10096), CIRCULATING DNA(D11096), TISSUE DNA (D20096), FFPE TISSUE DNA (D22096), MTB DNA (D23096), STOOL DNA (D24096), PLANT DNA (D30096)



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.)

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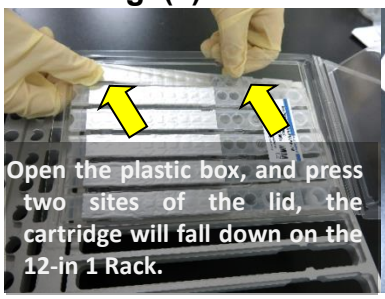
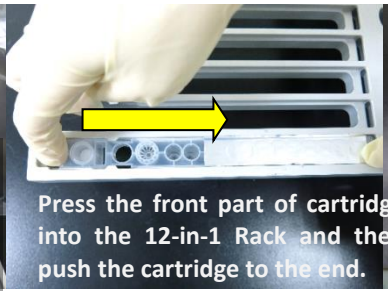
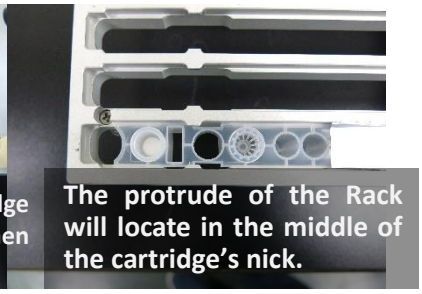
8. Load **Cartridge(s)** on the 12-in-1 Rack.

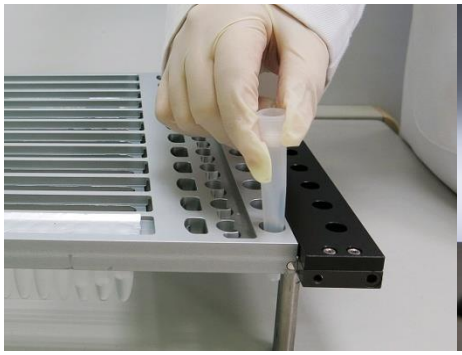






9. Place **Column** into the column position of cartridge.

10. Load **1ml 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.

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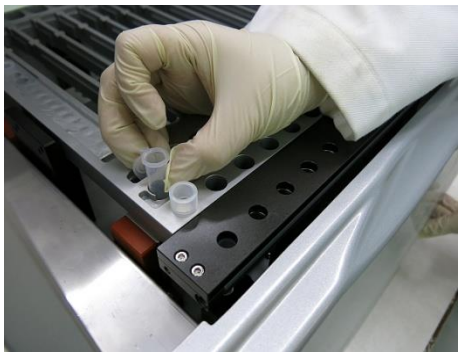
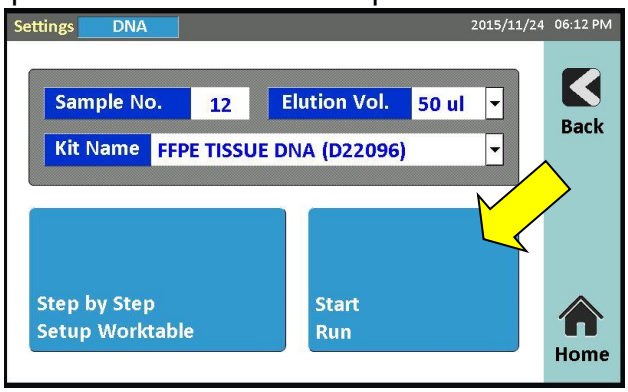
12. Place the 12-in-1 Rack into iColumn System and fix the 12-in-1 Rack by two lock plates aside the worktable.

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



15. Close the front door.
16. Tap "**Start Run**" to start the protocol.



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Sample Pretreatment

AccuPure Cell/Blood DNA Mini Kit (D10096)

◆ Please add 1100 µl Nuclease-free Water to 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℃ (1 year) or 4℃ (3 months)**.

I. For whole blood and body fluids

1. Add 20 µl Proteinase K into the bottom of the 2 ml Sample Tube.
2. Add 200 µl Whole blood and body fluid to the 2 ml Sample Tube.
3. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 mins at room temperature.
4. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Cell/Blood DNA (D10096)"**

II. For buffy coat

1. Centrifuge the whole blood at 2500 x g for 10 minutes at room temperature.
2. Pipette 20 µl Proteinase K into the bottom of the 2 ml Sample Tube.
3. Transfer up to 200 µl of the intermediate layer to the 2 ml Sample Tube.
4. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
5. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Cell/Blood DNA (D10096)"**

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III. For cultured cells

1. Harvest cells.
   A. Cells grown in suspension
      a. Transfer the appropriate number of cells (up to $1x10^6$) to a 1.5 ml micro-centrifuge tube (not provided).
      b. Centrifuge at 300 x g for 5 minutes.
      c. Remove the supernatant carefully and thoroughly.
   B. Cells grown in monolayer
      a. Detach cells from the dish or flask by trypsinization or by using a cell scraper.
      b. Centrifuge at 300 x g for 5 minutes.
      c. Remove the supernatant carefully and thoroughly.
2. Add 20 µl Proteinase K into the bottom of the 2 ml sample tube.
3. Resuspend cell pellet in PBS to a final volume of 200 µl. Transfer the cells with PBS into 2 ml Sample Tube.
4. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
5. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Cell/Blood DNA (D10096)"**

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- Please add 550 µl Nuclease-free Water to Proteinase K vial to make a **20 mg/ml** stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at -20℃ (1 year) or 4℃ (3 months)**.
- Add 1350 µl Nuclease-free Water to the tube containing 1350 µg lyophilized carrier RNA to obtain a solution of **1 µg/µl**. Dissolve the carrier RNA thoroughly, and divide it into conveniently sized aliquots (50-100 µl per tube), and **store it at -20℃**. **Do not freeze-thaw the aliquots of carrier RNA more than 3 times**.
- For Whole Blood, centrifuge the whole blood at 1,900 x g for 10 minutes at room temperature. Transfer upper layer to the 1.5 ml micro-centrifuge tube (not provided, please avoid aspirating any cell debris or WBC, and intermediate layer, otherwise it will co-extract gDNA from intact cells). And then centrifuge at≧ 11,000 x g for 10 minutes and transfer the supernatant for following extraction.
- For Whole Blood, plasma should be separated as soon as possible, and stored at -20 ℃, otherwise it will cause cfDNA degradation. Do not freeze-thaw the plasma repeatedly, otherwise it will degrade cfDNA and cause low DNA yield.
- For Plasma, Urine and Body Fluid, centrifuge at ≧ 11,000 x g for 10 minutes and transfer the supernatant for following extraction.

I. For plasma, urine and body fluid

1. Add 40 µl Proteinase K (20 mg/ml) into the bottom of the Screw Tube.
2. Add 10 µl carrier RNA into the bottom of the Screw Tube.
3. Transfer 800 µl of sample (already centrifuged with high speed) to the Screw Tube.
4. Add 800 µl of DL Buffer to the Screw Tube.
5. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **“Circulating DNA (D11096)”**

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◆ Please add 1100 µl Nuclease-free Water to 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℃ (1 year) or 4℃ (3 months)**.

I. General pretreatment 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 the following methods.
   A. Cut tissue samples into small pieces.
      Place the sample into a 2 ml micro-centrifuge tube (not provided). Proceed with step 3.
   B. 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.
   C. Homogenize tissue sample with buffer.
      Place tissue sample into 2 ml micro-centrifuge tube(not provided) containing 100 µl PBS. Homogenize samples with homogenizer thoroughly. Add 100 µl DATL Buffer and proceed with step 4.
3. Add 200 µl DATL Buffer and mix thoroughly by vortex. Spin down the sample.
4. Add 20 µl Proteinase K, and mix by vortex for 30 seconds. Spin down the sample.
5. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
6. Incubate at 60 ℃ for 10-20 minutes or until the tissue is completely lysed (vortex occasionally during incubation).
7. Centrifuge at 11,000 x g for 5 minutes.
8. Transfer 200 µl clear lysate to the 2ml Sample Tube. (Avoid aspirating any debris. If the sample is too thick, it may cause sample overloading. Please appropriately dilute the sample with DATL buffer.)
9. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

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II. For dried blood spot

1. Cut the dried blood spot into a micro-centrifuge tube (not provided).
2. Put 3 mm stainless bead*1 into micro-centrifuge tube.
3. Add 150 µl PBS and 150 µl DATL Buffer (For larger dimension of dried blood spot, add 200 µl PBS and 200 µl DATL Buffer.).
4. Homogenize samples with homogenizer thoroughly.
5. Add 20 µl Proteinase K, and mix by vortex for 15 seconds.
6. Incubate at 60 ˚C for 10 minutes.
7. Centrifuge at 11,000 x g for 1 minute.
8. Transfer 200 µl clear lysate to the 2 ml Sample tube.
9. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
10. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

III. For nails

1. Cut the sample (up to 25 mg) into small pieces and place the sample into a 1.5ml micro-centrifuge tube (not provided).
2. Add 20 µl 1M DTT (not provided) into the tube.
3. Proceed to step 3 of **General pretreatment for animal tissue**.

IV. For hair

1. Cut off a 0.5-1 cm length from the base of the hair, and transfer it to a 1.5ml micro-centrifuge tube (not provided).
2. Add 20 µl 1M DTT (not provided) into the tube.
3. Proceed to step 3 of **General pretreatment for animal tissue**.

V. For feathers

1. Cut a 2-3 cm piece from the feather** (up to 25 mg), and transfer it to a 1.5ml micro-centrifuge tube (not provided).

   **For small birds, such as finches, use primary feathers (e.g., the largest tail or wing feathers). For medium birds, such as canaries, secondary tail or wing feathers can be used. For large birds, such as chickens or cockatoos, breast or back feather can be used.
2. Add 20 µl 1M DTT (not provided) into the tube.
3. Proceed to step 3 of **General pretreatment for animal tissue**

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VI. For bacterial cultures

1. Transfer 1 ml well-grown bacterial culture (up to $1x10^9$ cells) to a micro-centrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at 11,000 x g for 2 minutes and discard the supernatant thoroughly.
3. Proceed to step 3 of **General pretreatment for animal tissue**

VII. For bacterial in body fluids

1. Collect bacteria by centrifuging biological fluids at 5,000 x g for 10 minutes.
2. Discard the supernatant thoroughly.
3. Proceed to step 3 of **General pretreatment for animal tissue**

VIII. For Gram-positive bacterial

A. Enzymatic method

1. Transfer 1 ml well-grown bacterial culture (up to $1x10^9$ cells) to a micro-centrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at full speed for 2 minutes and discard the supernatant completely.
3. Resuspend the cell pellet in100 µl lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCI, pH 8.0; 2 mM EDTA; 1.2 % Triton).
4. Incubate at 37 ˚C for 30 minutes.
5. Add 20 µl Proteinase K to the sample, and then add 100 µl DATL Buffer to the sample. Mix thoroughly by pulse-vortexing.
6. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
7. Incubate at 60 ˚C for 30 minutes and then for a further 15 min at 95 ˚C.
8. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **“Tissue DNA (D20096)”**

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B. Physical method

1. Transfer 1 ml well-grown bacterial culture (up to 1x10^9 cells) to a micro-centrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at full speed for 2 minutes and discard the supernatant completely.
3. Resuspend the cell pellet in 200 µl Buffer DATL, and transfer the sample into Beads Tube (optional). Mix thoroughly by pulse-vortexing for 30 seconds.
4. Incubation at 90˚C for 15 minutes.
5. Allow to cool at room temperature (15-25 ˚C). Add 20 µl Proteinase K to the sample and mix thoroughly by pulse-vortexing for 30 seconds.
6. Incubation at 60 ˚C for 15 minutes.
7. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
8. Centrifuging at full speed for 5 minutes.
9. Aspirate 200 µl supernatant into 2.0 ml Sample Tube.
10. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

IX. For Avian Blood

1. Transfer 10 µl avian blood into 2 ml Sample Tube.
2. Add 190 µl PBS to the sample.
3. Add 20 µl Proteinase K into the bottom of the 2 ml Sample Tube.
4. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
5. Spin down the sample. Proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

X. For Insect

1. Transfer the insect sample (up to 50 mg) to a micro-centrifuge tube (not provided).
2. Proceed to step 2 of **General pretreatment for animal tissue.**

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XI. For Urine

1. Centrifuge 5-50 ml sample at 13,000 x g for 10 minutes at room temperature.
2. Discard the supernatant. Add 200 µl buffer DATL to the sample then mix by vigorously vortex for 30 seconds. Transfer the sample with DATL into 2.0 ml Sample Tube.
3. Add 20 µl Proteinase K, and mix by vigorously vortex for 30 seconds.
4. (Optional) If there any visible particles or sandy debris in the pellet, centrifuge again and aliquot 200 µl supernatant for following steps.
5. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
6. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

XII. For Swab

1. Place the swab in a micro-centrifuge tube (not provided). Add 400 µl (cotton and DACRON) or 600 µl (Omni Swab) PBS to the sample.
2. Add 400 µl (cotton and DACRON) or 600 µl (Omni Swab) buffer DATL to the sample then mix by vigorously vortex for 30 seconds.
3. Add 20 µl Proteinase K, and mix by vigorously vortex for 30 seconds.
4. Incubate at 60 ˚C for 10 minutes. (Vortex twice during incubation.)
5. Centrifuge at 11,000 x g for 5 minutes.
6. Transfer 200 µl clear lysate to the 2 ml Sample tube. (avoid aspirating any debris)
7. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
8. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

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XIII. For LBC (Liquid-Based Cytology), ex: SurePath®, ThinPrep®

Normal

1. Add 80 µl DATL Buffer to 2 ml Sample Tube.
2. Transfer 250 µl liquid media to 2 ml Sample Tube. Vortex vigorously for 30 seconds and briefly spin down.
   *Avoid aspirating any mucus or debris while transferring sample.
3. Add 20 µl Proteinase K into 2 ml Sample Tube. Vortex vigorously for 30 seconds.
4. (Optional) Add 6 µl Carrier RNA into 2 ml Sample Tube. Mix by vortexing and briefly spin down.
5. Proceed to step 14 of **Operation Procedure**.

High Sensitivity

1. Centrifuge 1-10 ml sample at 5,000 x g for 10 minutes at room temperature.
2. Discard the supernatant. Add 200 µl DATL buffer to the sample then mix by vigorously vortexing for 30 seconds.
3. Add 20 µl Proteinase K, mix by vigorously vortexing for 30 seconds.
4. Incubate at 60 ˚C for 10 minutes.
5. Centrifuge at 11,000 x g for 5 minutes.
6. Transfer 200 µl clear lysate to the 2.0 ml Sample tube. (Avoid aspirating any debris.)
7. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
8. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

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XIV. For cytology brush in preserving tube

1. Check the medium volume of preserving tube, and add equal volume of DATL buffer into preserving tube then mix by vigorously vortexing for 30 seconds.
2. Incubate in 60℃ heat bath for 30 minutes.
3. Add 20 µl Proteinase K into the bottom of the 2 ml Sample Tube.
4. (Optional) 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. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

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XV. For saliva

Preservation solution with lysis buffer. Ex: DNA Genotek®

1. Mix the sample in the preservation solution by inversion and gently shaking for 30 seconds.
2. Incubate at 50℃ for 1 hour in the water bath or 2 hours by air incubator. (Or incubate at 50℃ overnight.)
3. Transfer 200 µl sample lysate into 2 ml Sample Tube.
   *Avoid aspirating any mucus or debris while transferring sample.
4. Add 20 µl Proteinase K, and mix by vigorously vortexing for 30 seconds.
5. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
6. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

Preservation solution without lyse buffer.

1. Centrifuge 1-10 ml sample at 5,000 x g for 10 minutes at room temperature.
2. Discard the supernatant. Add 100 µl PBS Buffer to suspend the pellet.
   *If it is possible to do 2nd or 3rd repeats test, please enlarge the input volume of PBS buffer and utilize 100 µl samples for each run of purification.
3. Add 100 µl buffer DATL to the sample then mix by vigorously vortexing for 30 seconds.
4. Add 20 µl Proteinase K, mix by vigorously vortexing for 30 seconds.
5. Incubate at 60 ℃ for 10 minutes or until the tissue is completely lysed (Vortex occasionally during incubation.).
6. Centrifuge at 11,000 x g for 5 minutes.
7. Transfer 200 µl clear lysate to the 2.0 ml Sample tube.
   *Avoid aspirating any mucus or debris while transferring sample.
8. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
9. Briefly spin down, and proceed to step 14 of **Operation Procedure** and tap **"Tissue DNA (D20096)"**

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◆ **PK Plus stock solution for D22096**

#Add 1100 µl PK Plus Solution to PK Plus vial to make a **20 mg/ml** stock solution. Vortex and make sure that PK Plus has been completely dissolved. **Store the stock solution at -20℃ (1 year) or 4℃ (3 months)**.

I. For FFPE Samples.

1. Place 5-10 µm sections (up to 5 sections) in the 1.5 ml micro-centrifuge tube (not provided).
2. Add 500 µl DWX buffer, vortex or ranking vigorously for 30 seconds. Spin down to collect sample in the bottom.
3. Incubate at 90℃for 20 minutes. After incubation, cool down the samples at room temperature (15-25℃).
4. Add 300 µl DATL Buffer and mix thoroughly by vortexing.
5. Centrifuge at 9,600 x g for 1 minute.
6. #Add 30 µl PK Plus to the lower clear phase. Mix gently by pipetting.
7. Incubate at 56℃ for 1-2 hours or until the tissue is completely lysed.
8. Centrifuge at 9,600 x g for 1 minute.
9. Transfer the 300 µl lower clear phase into a new 2 ml Sample tube.
10. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
11. Briefly spin down, and proceed to step 14 of Operation Procedure and tap “**FFPE Tissue DNA (D22096)”**

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◆ Please add 1100 µl Nuclease-free Water to 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℃ (1 year) or 4℃ (3 months)**.

I. For MTB Samples.

A. Liquidize Sputum for Cell culture

1. Add 0.9 ml Sputum into 50 ml Conical Tube.
2. Add 0.9 ml NALC-NaOH. Mix by vortexing for 15-20 seconds and spin down.
3. Incubate at room temperature for 15 minutes.
4. Add PBS to 40 ml.
5. Centrifuge at 3000 g for 15 minutes.
6. Discard the supernatant carefully. Do not remove any of the pellets.
7. Add 1 ml PBS and mix by vortexing.
8. Incubate at 90 ˚C for 20 minutes, and the briefly spin down.

B. Sample pretreatment of iColumn

9. Transfer 100 µl inactivated MTB sample into a Screw Tube.
10. Add 200 µl DATL buffer and vortex for 30 seconds and spin down.
11. Add 25 µl Proteinase K and vortex for 30 seconds and spin down.
12. (Optional) If RNA-free genomic DNA is required, add 4µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
13. Sonicate at 56℃ for 10 minutes (The sonicator was not provided.).
14. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
15. Briefly spin down, and proceed to step 14 of Operation Procedure and tap **“MTB DNA (D23096)”**

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- Please add 1100 µl Nuclease-free Water to 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℃ (1 year) or 4℃ (3 months)**.

I. For Stool for Pathogen Detection

1. Weight 25 mg (up to 50 mg) stool or sample into the Beads Tube.
2. Add 1000 µl DSTL Buffer into the Beads Tube. Vortex continuously for 30 seconds or until the stool sample is thoroughly homogenized.
3. Incubation at 95℃ for 15 minutes.
4. Centrifuge at 11.000 x g for 5 minutes.
5. Aliquot 200 µl supernatant to the 2.0 ml Sample Tube.
6. Add 20 µl Proteinase K into 2.0 ml Sample Tube.
7. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
8. Briefly spin down, and proceed to step 14 of Operation Procedure and tap "**Stool DNA (D24096)"**

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IVD

I. General pretreatment for plant and fungi tissue (Ex fungi, corn, rice, tobacco, wheat etc.)

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue or 10 mg (up to 20 mg) of dried sample.

   (If the plant or fungi grown on the medium, please heat the sample to 80˚C. Discard the medium, and wash by PBS for several times. Remove liquid as much as possible.)
2. Homogenize tissue samples by one of following methods.
   A. <u>Homogenize with liquid nitrogen.</u>
      a. Use homogenizer (ex. Tissue Lyser, Beads Beater etc.)
         i. Grind tissue sample thoroughly (2 mm x 2 mm) and place it into 2.0 ml micro-centrifuge tube (not provided).
         ii. Add appropriate amount (1-3 beads) and the appropriate size (3-5 mm) of stainless beads.
         iii. Place the sample tube into liquid nitrogen, incubation least 2 minutes.
         iv. Homogenized the sample tube at 30Hz for 30 seconds by beads beater.
         v. Remove the sample tube and check whether the sample became homogeneous powder, and store the sample back into liquid nitrogen ice as soon as possible.
         vi. If the sample is not homogeneous, repeat steps iii, iv, v, lead the sample homogeneous into powder completely.
         vii. Add 450 µl DPTL1-S Buffer and mix by vortexing for 30 seconds immediately.
      b. Use mortar & pestle
         i. Add appropriate volume of liquid nitrogen into mortar & pestle.
         ii. Place the sample into the mortar.
         iii. Add liquid nitrogen to grind the sample powder.
         iv. Load sample powder into the sample tube pre-cooled by liquid nitrogen.
         v. Add 450 µl DPTL1-S Buffer and mix by vortexing for 30 seconds immediately.

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B. Homogenize tissue sample with buffer.

i. Grind tissue sample thoroughly (2 mm x 2 mm) and place it into 2.0 ml micro-centrifuge tube (not provided).

ii. Add appropriate amount (1-3 beads) and the appropriate size (3-5 mm) of stainless beads.

iii. Add 450 µl DPTL1-S Buffer and homogenize by homogenizer.

3. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate 2 minutes at room temperature.
4. Incubate at 65 ˚C for 10-20 minutes. (Vortex the tube every 3-4 minutes during incubation)
5. Add 150 µl DPTL2-S Buffer and mix by vortexing for 30 seconds.
6. Incubate on ice for 5 minutes.
7. Centrifuge at 11,000 x g for 5 minutes and transfer 400 µl clear lysate to the 2.0 ml Sample tube. (avoid aspirating any debris)
8. Briefly spin down, and proceed to step 14 of Operation Procedure and tap "**Plant DNA (D30096)"**.

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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 DNA purification procedure with a new sample. Be sure to add proper amount of Proteinase K.
Inefficient cell lysis due to decreased activity of Proteinase K.	Stop the automatic system and repeat the DNA purification procedure with a new sample. Ensure that Proteinase K stock solution is store at -20℃.
1-2. Sample is not free from solid impurities due to improper sample pretreatment.	Stop the automatic system and repeat the DNA purification procedure with a new sample. Ensure to follow sample pretreatment guide according to different samples.
2. Little DNA 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 DNA 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 DNA, 50 μl of elution buffer is recommended.
2-3. Inefficient cell lysis due to decreased activity of Proteinase K.	Repeat the DNA purification procedure with a new sample. Ensure that Proteinase K stock solution is store at 2-8 ℃.
3. A260/A280 ratio for purified DNA is low.	
3-1. Sample is not fresh due to too long maintenance.	Use fresh or properly stored sample and Repeat the DNA purification procedure.
3-2. Inefficient cell lysis due to decreased activity of Proteinase K.	Repeat the DNA purification procedure with a new sample. Ensure that Proteinase K stock solution is store at 2-8 ℃.

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CE IVD

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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IVD

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