

Isolation of cell free DNA **Quick Guide**

ver.0.1

QuickGene cfDNA Isolation Kit (QG CF-L)

In this Quick Guide, the protocol for isolation of cfDNA from plasma is a digest from the Handbook of QuickGene cfDNA Isolation Kit (QG CF-L) and the Operation manual of QuickGene-Auto240L.

* Before using, please read the Operation manual.



Wear protective gloves and safety goggles during the experiments.

Step1 Preparations

In order to isolate the target cfDNA, please prepare the following items.

1 Accessories

QuickGene-Auto240L (240L)		Reagent tip holder		Waste container	
Operation manual		Sample holder x3		Wash buffer bottle	
Power cable		Cartridge holder x3		Reagent container S x3	
Reagent container holder		Waste tube holder x3		Reagent container L (Waste fluid container) x4	
Sample tip holder		Collection tube holder x3			

2 Consumables

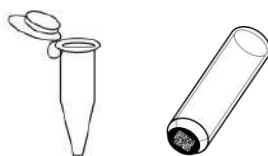
cfDNA Isolation Kit (QG CF-L)

- Cartridge x48
- Waste tubes x48
- Reagents

QuickGene-Auto240L Consumables Kit (QG-240L-CK)

- Lysate tube x48
- 10 ml tip x60
- 1.2 ml tip x96

Collection tube (1.5mL Microtube or 2D barcoded Matrix™ tube 1.4ml)



Sample tube

<Recommended product>

Falcon® 5 mL Round Bottom Polystyrene Test Tube, without Cap, Nonsterile, 1000/Bag, 1000/Case (#352008, CORNING)

>99% Ethanol

Nuclease free water

Protective gloves

Safety goggles

3 Preparations of reagents

◆ **Protease (ECF-01)**

Add 3.3ml nuclease-free water into the vial containing lyophilized Protease, leave it for 30 min or more at room temperature with occasionally stirring it. Dissolve it completely.
(Store the dissolved protease (ECF-01) at 4°C. The dissolved protease (EDB) will be able to store for two months at 4°C. The enzyme will be stable for a longer period at -20oC. Recommend to avoid repeated freezing and thawing.)

◆ **Lysis Buffer (LCF-01)**

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubation at 37°C.

◆ **Wash Buffer (WCF-01)**

Add 160ml ethanol (>99%) into the bottle and mix well.
After adding the ethanol, close the cap and store at room temperature.

◆ **Elution Buffer (CCF-01)**

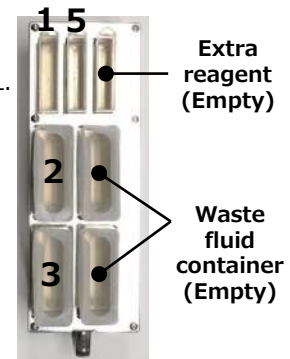
Use CCF-01 for elution of cfDNA.

4 Setting of reagents

◆ **Reagent container and necessary reagents volume**

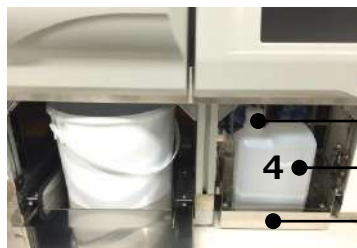
Reagents	Container for 240L	Reagent set position	Volume / Sample	Other Necessary Volume	Necessary volume per operation		
					8 sample	16 sample	24 sample
ECF-01	Reagent container S	1	0.3 ml	1 ml	3.4 ml	5.8 ml	8.2 ml
LCF-01	Reagent container L	2	2.5 ml	10 ml	30 ml	50 ml	70 ml
>99% Ethanol	Reagent container L	3	1.2 ml	10 ml	20 ml	29 ml	39 ml
WCF-01 (Ethanol mixed)	Wash buffer bottle	4	19.5 ml	50 ml	206 ml	362 ml	518 ml
CCF-01	Reagent container S	5	0.1 ml	1 ml	1.8 ml	2.6 ml	3.4 ml

- 1) In reference to the above table, transfer the necessary volume of the reagents into Reagent containers for 240L.
- 2) Set the Reagent container S and L to the Reagent container holder.
- 3) Set the Reagent container holder to the Reagent container holder slot in 240L.



- 4) Set the Wash buffer bottle into the Wash buffer bottle rack of the 240L drawer.

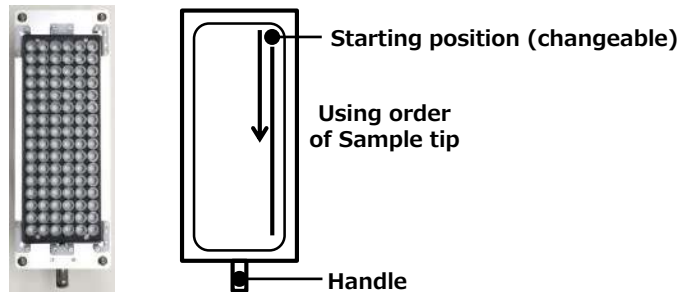
Please confirm that the two inlet tubes are reached to the bottom of the Wash buffer bottle.



5 Setting of consumables

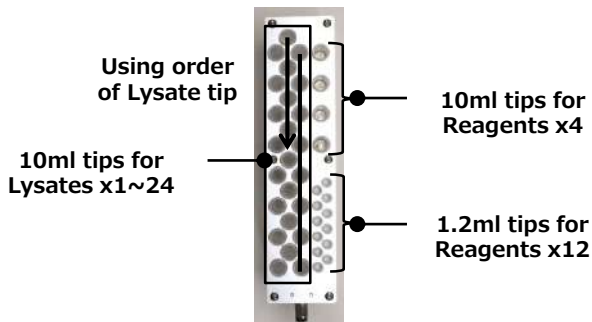
- 1) Set the 1.2ml tip rack to the Sample tip holder.

Confirm that the tip number is same or more than sample number.

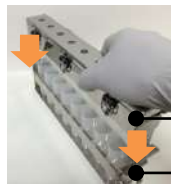


- 2) Set the 1.2ml and 10ml tips to the Reagent tip holder.

**Set all the tips for Reagents (1.2ml x12, 10ml x4).
Set the tips of same or more than sample number for Lysates (10ml x1~24).**

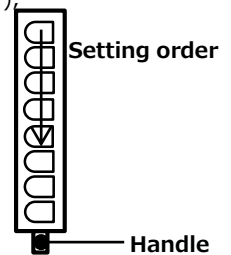


- 3) Set the Waste tubes to the Waste tube holder (same number as sample number),
and set the Cartridge holder on the Waste tube holder.



Cartridge holder

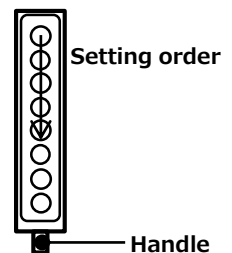
Waste tube holder



- 4) Set the Cartridges to the Cartridge holder (same number as sample number),
and close the cover and lock the 3 fasteners.



Lock
the 3 fasteners



- 5) Set the Collection tubes to the Collection tube holder (same number as sample number).

Use the adaptor to fit the size of Collection tubes.



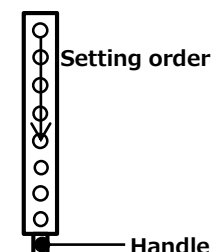
1.5ml microtube
(without adaptor)



Adaptor



2D barcoded MatrixX™
tube
(with adaptor)



6) Set all the holder to each holder slot in 240L.

- (4) Sample tip holder (1), Reagent tip holder (2), Cartridge/Waste tube holder (3), Collection tube holder (4)

If the Collection tubes have barcodes, set the Collection tubes to the fixed slot according to the "Step2 Operation".

7) Open the Agitator cover and set the Lysate tubes (5) on the Lysate unit in the 240L (same number as sample number). After setting the Lysate tube, close the Agitator cover tightly.

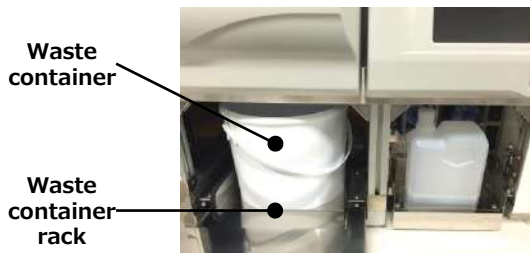


4 3 5 Sample holder slot 2 1 Reagent container holder slot



8) Set the Waste container into the Waste container rack of the 240L drawer.

Confirm that the Waste container is empty.



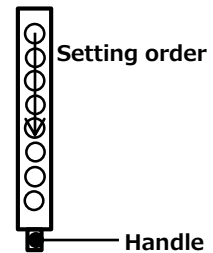
6 Sample preparation and setting

- 1) Mix the sample of sample tube by inverting gently.
- 2) Remove the lid of sample tube
- 3) Set the sample tube to the Sample holder.

Use the adaptor to fit the size of sample tubes.



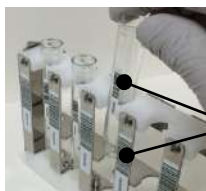
Adaptor



4) Set the Sample holder to the Sample holder slot in 240L.

If the sample tube have barcodes,
 - set the barcode side of sample tube to the same side of barcode on the Sample holder.
 - set the Sample holder to the fixed slot according to "Step2 Operation".

After setting, start the operation of 240L within 10 minutes.



Side of barcode



Continue to Step2

Step2 Operation

In order to get the target yield of DNA, please follow the protocol below.

The elution volume from each Cartridge is 100µl.

Isolate a plasma as soon as possible after blood collection. Otherwise, genomic DNA from blood cell is increased.

1 Set the holders and Waste container.

Refer to “Step1 Preparations”.

In case of barcode reading mode, set the Sample holder and Collection tube holder in later steps.

2 Turn the Power ON and proceed to the “MODE SELECT” screen.

- 1) Check the power cable connection and the earth leakage breaker.
- 2) Confirm that the slide door, flap doors and drawer are closed.
- 3) Press the Power switch ON (located below of the Operation panel).

Wait until the doors are locked and the “SYSTEM CHECK” button is activated.



- 4) Press the “SYSTEM CHECK” button and wait until the checks are completed.



- 5) After all the checks are complete, press “OK” button.

If some items are “NG”, refer to the Operation manual and solve the matters.



- 6) After select the “USER ID” and input “USER PASSWORD”, press “SIGN IN” button.

Refer to the Operation manual about “USER ID” and “USER PASSWORD” setting.

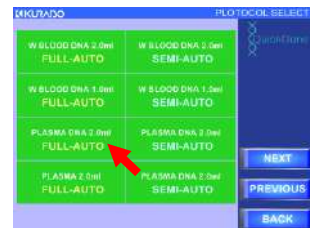


3 Setting of barcode reading mode

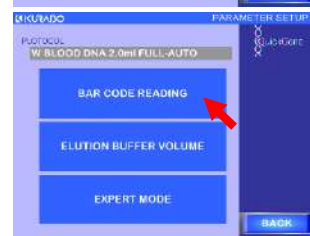
1) Press the "PARAMETER SETUP" button on the "MODE SELECT" screen.



2) Select and press the protocol button (ex. "PLASMA DNA 2.0ml FULL-AUTO").



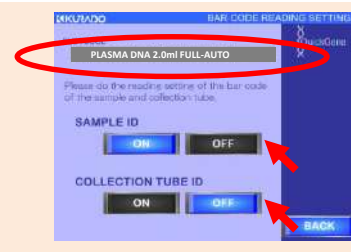
3) Press the "BARCODE READING" button.



4) "Choose ON/OFF of "SAMPLE ID" and "COLLECTION ID".

Barcode reader for Collection tube is optional. You can select the 3 patterns of ID setting.

Pattern	Sample ID	Collection ID
1	ON	ON
2	ON	OFF
3	OFF	OFF



5) Press the "BACK" button, and back to "MODE SELECT" screen.

4 Operation - Protocol select

1) Press the "AUTOMATED OPERATION" button on the "MODE SELECT" screen.



2) Press the "OK" button on the pop-up window.

Confirm that the Waste container in the drawer is empty.



3) Select and press the protocol button

(ex. "PLASMA DNA 2.0ml FULL-AUTO").

5 Operation – Sample set / Sample ID check

SAMPLE ID “ON”

- 1) After the doors are unlocked, open the Right flap door.
- 2) Set the Sample holder A to the Sample holder slot A slowly to read the barcodes.

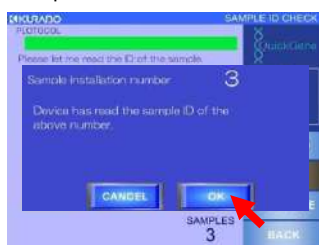
Set the barcode side of sample tube to the same side of barcode on the Sample holder.



- 3) Confirm that the detected number is correct to your sample number. If you have more sample, press “NEXT” button and set the Sample holder B and C with same way as holder A.
- 4) After all the samples are detected and set, press the “COMPLETE” button.



- 5) After confirming the sample number displayed on the pop-up window, close all the doors and press the “OK” button.



SAMPLE ID “OFF”

- 1) Press the button for your sample number.
- 2) Press the “OK” button.



6 Operation – Collection ID check***Barcode reader for Collection tube is optional.****COLLECTION ID “ON”**

1) Open the Left flap door, insert the Collection tube holder A to the Matrix tube barcode reading slot, and read barcodes slowly.

If the position of Collection tubes and that of samples are matched, pop-up window is shown.



2) Set the Collection tube holder A to the Collection tube holder slot A.

If the Collection tube holder is set to the correct slot, pop-up window disappears.

3) If you have more Collection tubes, press “NEXT” button. And read the barcodes and set the Collection tube holder B and C to the slots with same way as holder A.

4) After all the Collection tubes are detected and set, press the “COMPLETE” button.

If all the position of Collection tubes and that of samples are matched, the “COMPLETE” button is activated.



5) After confirming the sample number displayed on the pop-up window, close all the doors and press the “OK” button.

**COLLECTION ID “OFF”**

If you choose Collection ID “OFF”, proceed next screen automatically.

7 Operation – Reagent confirmation

- 1) See display screen, and confirm that all the Reagents have enough volume and are set to the correct position.
- 2) Press the "CHECK" buttons for each Reagents or press the "ALL" button

When all the "CHECK" button are pressed, the "OK" button is activated.

- 3) Press the "OK" button.



8 Operation – Start mode select

- 1) Set the starting position of Sample tip. If you want to start from first position, press the "RESET" button. If you want to start from other position, press the "CHANGE" button and input any number.

Sample tip position is memorized in the instrument from operation history.

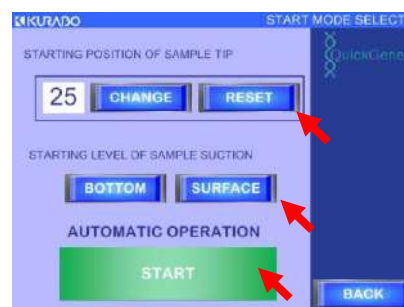
- 2) Choose the starting level of sample suction. If you want to suction samples from bottom of sample tube, press the "BOTTOM" button. If you want to suction samples from the each surface, press the "SURFACE" button.

Only when your sample volume is less than 3ml, choose "BOTTOM" mode. When you choose "BOTTOM", overflow caution pop-up window is shown. Press "OK" and return to "START MODE SELECT" screen.



- 3) Press the "START" button and start the DNA isolation.

After completion of "SETTING CHECK" of each parts by sensors, isolation operation starts. If some items are "NG" at "SETTING CHECK", refer to the Operation manual and solve the matters.



9 Automated Operation

Automated operation continue unless any serious errors occur. If any serious errors occur, refer to the Operation manual and solve the matters.

Continue to Step3

Step3 Collection and post-treatment

1 Operation results confirmation

- 1) The display screen (right) shows the completion of isolation operation. The color of sample position shows the isolation results.

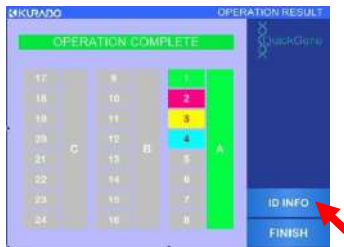
Color	Operation Result
Green	Normal End
Red(NG1)	Chip Clogging at Sample Suction
Yellow(NG2)	Pressure Leakage of Cartridge
Blue(NG3)	Clogged Cartridge
Gray	No Sample



About isolation failure (NG1 to 3), refer to the Operation manual and analyze the factors.

- 2) When you press "ID INFO" button, you can confirm SAMPLE ID and COLLECTION ID information. After confirming, press "BACK" button and return to the operation result screen.

"ID INFO" button is activated only when the SAMPLE ID is "ON".
When the COLLECTION ID is "OFF", the COLLECTION ID information correspond to sample position (ex. A-1, A-2, ...).



- 3) Press "FINISH" button on the operation result screen.
4) Press "OK" button on the pop-up window.



2 DNA sample collection

- 1) Turn the Power switch OFF.
- 2) Open the Left flap door and take the Collection tube holder out.
- 3) Close the Caps of the Collection tubes tightly.

In case storing genomic DNA for a long time, preserve at -20°C .
The default volume of Elution Buffer (CCF-01) is 100 μl .

3 Consumables and waste disposal

- 1) Take all the holder and consumables out of the 240L.
 - Cartridge/Waste tube holder, Sample tube holder, Lysate tube, Waste container
- 2) Remove the Cartridge, Waste tube and Sample tube from their holder.
- 3) Dispose the wastes and the waste fluid according the applicable regulation.
 - Cartridge, Waste tube (waste fluid), Sample tube, Lysate tube, Waste container (waste tips)



BIO HAZARD

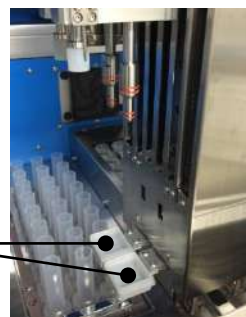
Dispose of the infectious waste according to your governments requirement.

4 Post-treatment

- 1) Check the Drip tray. If waste remains on them, dispose and clean it.

About cleaning, refer to the Operation manual.

Drip
tray



- 2) Clean each parts and holders.

- 240L working area, Pressurization packing, Drip tray, Cartridge holder (Holder packing), Waste tube holder, Sample holder, Collection tube holder, Reagent container holder, Sample tip holder, Reagent tip holder

- 3) If necessary, irradiate the UV inside of 240L.

About the "UV IRRADIATION MODE", refer to the Operation manual.



- 4) After operation

- If continue the operation,
⇒ Start from "Step1 Preparations".
- If the 240L will not be used for a week or more,
⇒ Clean the Wash buffer line, and shut the 240L down.

About cleaning of Wash buffer line, refer to the Operation manual.

5 Confirmation of operating history

1) Press the "OPERATING HISTORY" button on the "MODE SELECT" screen.



2) Operating information displays.

- Operating No. (Max 100 operations), Date/Time, User ID, Details

When the SAMPLE ID / COLLECTION ID is "OFF", the ID information correspond to sample position (ex. A-1, A-2, ...).



3) Press the "VIEW" button.

4) Press the "ID INFO" button.



5) ID information displays.

- Sample No., Operation result, Sample ID, Collection ID



6 Data Saving of Operating information

1) Press the "DATA SAVE" button on the "OPERATING HISTORY" screen, and press the "OK" button on the pop-up window.



2) After selecting the data to press the "No.", "PAGE SELECT", or "ALL SELECT" button, press the "COMPLETE" button.



3) Plug in a USB memory stick into the port 1 or 2 on the side of 240L.

4) Press the "SAVE" button.



5) Plug out the USB memory stick from the port.