KURABO

HANDBOOK

QuickGene cfDNA isolation kit (CF-L)

Ver.1.0

Contents

Warning For research use only. Not recommended and intended for diagnostic or clinical application for human and animals.

1. Introduction

QuickGene porous membrane to immobilize nucleic acid has large specific surface area and uniform & fine porousness. So QuickGene successfully extracts cell-free DNA (cfDNA) with high yield; moreover, with its patented thin membrane, it eliminates most contaminants.
QuickGene also uses pressured filtration technology, which cannot be successfully utilized with typical glass membranes; by using pressured filtration technology, new, compact and automatic instruments for rapid nucleic acid purification can be produced successfully.

○cfDNA from plasma samples can be simultaneously extracted in following time.

・QuickGene-Auto240L (QG-Auto240L):about 170 min for 24 sets of plasma sample

○cfDNA from lysate can be extracted simultaneously at the following time

・QuickGene-Mini8L (QG-Mini8L):about 80 min for 8 sample

2. Kit components and storage conditions

2-1. Kit components

Confirm that following contents is packed.
There are 48 reactions of cfDNA isolation reagent and consumables.

Name	Number	Volume
Protease (ECF)	5 vials	Lyophilized
Lysis Buffer (LCF)	2 bottles	70 ml
Wash Buffer (WCF)	4 bottles	160 ml
Elution Buffer (CCF)	1 bottle	100 ml
Cartridges (CAL2)	1 bag	48 pcs
Waste Tubes (WT)	1 bag	48 pcs

2-2. Storage conditions

All reagents are stable at room temperature (15-28°C) until expiring date indicated at outer box. The dissolved protease (ECF) will be able to store for two months at 4°C.
Storage at -20°C will prolong the life of ECF, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at -20°C is recommended.

3. Other required materials, not supplied in this kit

[1] Reagent

- >99% Ethanol (for Molecular Biology / Purity:≧99.5%)
- Nuclease-free ultrapure water (for dissolving protease)

[2] Equipment

- QG-Auto240L or QG-Mini8L

- 50 ml or 15 ml of centrifuge tube (*)
- Micropipette and tips
- 1.5 ml microtube (used for collection tube of isolated DNA reagent) (**)
- Tube stand

(*) : 15 ml (or 50 ml) centrifuge tube is used for sample pre-treatment. 50 ml centrifuge tube is used as CCF container.

(**) : Microtube with ball rock cannot be used.

[3] Additional required materials when using QG-Mini8L

- Tube mixer
- Table top water bath (for incubation of 50 ml or 15 ml centrifuge tubes at 56°C)

[4] Additional required materials when using QG-Auto240L

- Sample Tube (***)

  Size of Blood collection tube 6 mL: φ13 x 100 mm
  10 mL: φ16 x 100 mm
- Matrix tube (****)

  *Matrix™ 2D Barcoded Open-Top Storage Tubes Cat. No. 3791, 3792
- QuickGene Auto240L Consumables Kit (240L-CK)

(***) : Collect plasma in sample tube with a suitable blood collection tube size.

(****) : Not required if 1.5ml microtube is not used

4. Safety warnings

All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, disposable gloves and safety goggles during the experiments are highly recommended. In case of contact between the reagents and the eyes, skin, or clothing, wash immediately with water.
(See the Safety Data Sheet for specific recommendations, https://www.kurabo.co.jp/bio/English/)

◆ Protease ECF

- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ LCF (Lysis Buffer)

- Harmful if ingested.
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ WCF (Wash Buffer)

- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ CCF (Elution Buffer)

- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

- In the case of using and disposing potentially infectious samples and consumables
  Wear a suitable laboratory coat, disposable gloves and safety goggles during the experiments. After use, dispose of potentially infectious samples and consumables by incineration, high temperature decontamination, sterilization, or disinfection in accordance with applicable laws. When entrusting waste disposal to licensed hazardous waste disposal contractors, use specially controlled waste management forms (manifest), if applicable.

5. Precautions

- Corresponding sample: Plasma sample collected from whole blood sample treated with EDTA-2Na, EDTA-2K, or sodium citrate.
- Prepare plasma samples immediately after collect from whole blood.
  Whole blood cells are destroyed and the amount of genomic DNA increases. If you collect the plasma from whole blood is consuming, use a blood collection tube for cfDNA isolation.
- Use the plasma sample as much early as possible.
- Small amount of samples should be adjusted to 2 ml with PBS (sterilized) before loading.
- All operations should be performed at room temperature (15 to 28°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Keep away the Lysis Buffer (LCF) from heat (more than 28°C). Do not mix with disinfectants such as bleach.
- Default elution volume is 100 µl. In case of setting to less than 100 µl, yield may decline.

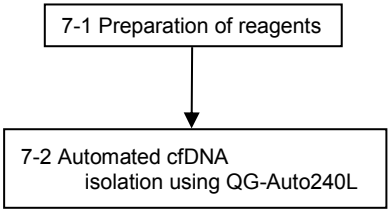
6. Quality controls

As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene cfDNA isolation kit (CF-L) is evaluated routinely on a lot-to-lot uniformity.

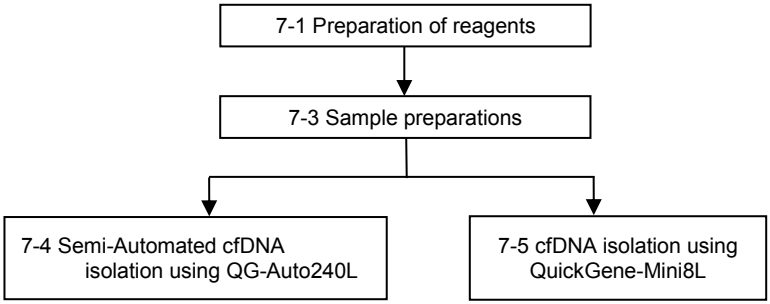
Yield and quality of cfDNA are checked by microchip electrophoresis.

7. Protocols

【Overview Flow Chart① –In case of QG-Auto240L-Automated- 】



【Overview Flow Chart② –In case of QG-Auto240L-Semi-Automated, QG-Mini8L】



7-1. Preparation of reagents

◆Protease (ECF)

When using ECF, pipette 3.3 ml of nuclease-free water into the vial containing lyophilized Protease. Dissolve it completely. Reconstituted ECF is stable for 2 months when stored at 4°C.
Storage at –20°C will prolong the life of ECF until 2 months, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20°C is recommended.

Notices Dissolve ECF completely by the following method, and then use the solution. Add 3.3 ml of nuclease-free water, close the cap and mix with inversion the bottle. Leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CAL2) might clog when dissolution of ECF is insufficient.

◆Lysis Buffer (LCF)

Mix thoroughly before using. If the precipitates are contained in Lysis Buffer (LCF), incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Lysis Buffer (LCF), cool down the bottle to room temperature before using.

◆Elution Buffer (CCF)

Make sure to use CCF for DNA elution.

◆Wash Buffer (WCF)

Provide the concentrated solution. Add 160 ml of >99% ethanol into the bottle and mix with inversion the bottle gently at the beginning of use.

7-2. Automated cfDNA isolation using QG-Auto240L

- Refer to the User's guide of QG-Auto240L and make necessary preparations.
- Waste tube, Cartridge and Collection tube should be set at the correct position with following the order.
- Set Waste tube, Cartridge and Collection tube at the correct position.

<1> (Set of reagent) Transfer the necessary volume of the reagents into Reagent containers for 240L. Set the reagent containers S and L with reagent in them in the reagent container holder according to the setting position numbers. Set an empty container at the setting position numbers for reagent containers not to be used and waste containers. Set the Reagent container holder to the Reagent container holder slot in 240L. Set the Wash buffer bottle into the Wash buffer bottle rack of the 240L drawer.

<2> (Preparation of consumables/accessories) Set the 1.2ml and 10ml tips to the Reagent tip holder. Set the Waste tubes to the Waste tube holder (same number as sample number), and set the Cartridge holder on the Waste tube holder. Set the Cartridges to the Cartridge holder (same number as sample number), and close the cover and lock the 3 fasteners. Set the Collection tubes to the Collection tube holder (same number as sample number).

<3> (Set of holders and waste containers) Set all the holder to each holder slot in 240L. Set the Waste container into the Waste container rack of the 240L drawer. Use the specific container and confirm that the Waste container is empty before use. The use of container not included in the delivery, use of un-emptied container or use with an erroneous setting may cause waste overflow.

<4> (Set of lysate tube) Open the Agitator cover and set the Lysate tubes on the Lysate unit in the 240L (same number as sample number). After setting the Lysate tube, close the Agitator cover tightly.

<5> (Sample preparation) Place plasma in sample tube with a suitable blood collection tube size, and set the sample tube in the sample tube holder. For details on how to prepare plasma, refer to "p.14 Appendix Preparation of plasma". Set the sample tube holder set in the QG-Auto-240L.

<6> (Isolation operation) Refer to the User's guide of QG-Auto240L "3.6 Start-up of System", turn on the power and proceed the mode select screen. Select "AUTOMATED OPERATION" and then select "PLASMA DNA 2ml FULL-AUTO". Press the numeric button corresponding to the number of set samples. Check that the selected button coincides with the number of set samples and press [OK]. Refer to the indicated information on the screen regarding the reagents to be used for the automatic isolating operation and confirm that the required quantity is set in the correct position. Press "CHECK" button of confirmed reagent and then press "OK" button. Automated operation is started when pressing "START" button.

<7> (Finish operation/ Collect DNA) The operation ends when the “AUTOMATIC OPERATION END” is displayed. Turn the Power switch OFF. Open the Left flap door and take the Collection tube holder out. Close the Caps of the Collection tubes tightly.
If you do not use DNA immediately, please close the tube lid tightly and store at 4°C or -20°C. In case storing cfDNA for a long time, preserve at -20°C.

<8> (Clean up)
Remove Cartridge Holder and Waste tube Holder. Dispose the Cartridges, Waste tubes and waste fluid according to applicable regulations.
Refer to the User’s guide of QG-Auto240L “3.9 Disposal of Consumables and Waste”.

7-3. Sample preparations

- The QuickGene cfDNA isolation kit is specifically designed for cfDNA isolation from 2 ml of plasma. Recommend using plasma sample collected from whole blood sample treated with EDTA-2Na, EDTA-2K, or sodium citrate.
- Please keep the below sample volume. Excess amount of sample may occur low isolation efficiency or interference with device operation.

1. Add 3.3 ml of nuclease-free ultrapure water to the vial containing the freeze-dried protease, and dissolve it carefully. Put the 300 µl of ECF to bottom of 15 ml tube.
   You can use 50ml centrifuge tube instead of 15ml tube.

2. Add 2 ml of plasma into the 15 ml tube.
   After adding plasma, proceed to step 3 and 4 immediately.
   Process one by one for step 3 and 4.

3. Mix the sample and LCF with shaking 10 times up and down.
   It is very important to mix thoroughly the sample after addition of LCF.

4. Vortexing for 15 sec. with maximum speed. Recommending vortex speed is 2,500 rpm and more.
   Incomplete mixing at this time, the sample will be clogged the cartridge, or low yield.

5. Incubate with water bath at 56°C 5 min. The maximum incubation time is 10 min.
   The incubation time should be adjusted depending on the speck of constant temperature bath. If actual liquid temperature is lower than 56°C, adjust the setting value to make the liquid temperature at 56°C.
   For example, in case of using the heating block, you have to incubate at 56°C 30 min.

6. Process this step one by one.
   Add 1.2 ml >99% Ethanol and mix the sample with shaking 10 times up and down. Mixing with the lysate and ethanol immediately is quite important. Mix by vortexing with holding around cap of 15 ml tube. vortexing for 15 sec. with maximum speed. Recommending vortex speed is 2,500 rpm and more. Incomplete mixing at this time, the sample will be clogged the cartridge, or low yield. Perform the isolation operation within 30 minutes, after completing the lysis.

7-4. Semi-Automated cfDNA isolation using QG-Auto240L

- Refer to the User's guide of QG-Auto240L and make necessary preparations.
- Waste tube, Cartridge and Collection tube should be set at the correct position with following the order.
- Refer to "4 Operation Semi-Automated protocol" in User's guide of QG-Auto240L for details.

Semi-automated isolation protocol with QG-Auto240L

＜1＞ (Set of reagent) Transfer the necessary volume of the reagents into Reagent containers for 240L. Set the reagent containers S and L with reagent in them in the reagent container holder according to the setting position numbers. Set an empty container at the setting position numbers for reagent containers not to be used and waste containers. Set the Reagent container holder to the Reagent container holder slot in 240L. Set the Wash buffer bottle into the Wash buffer bottle rack of the 240L drawer.

＜2＞ (Preparation of consumables/accessories) Set the 1.2ml and 10ml tips to the Reagent tip holder. Set the Waste tubes to the Waste tube holder (same number as sample number), and set the Cartridge holder on the Waste tube holder. Set the Cartridges to the Cartridge holder (same number as sample number), and close the cover and lock the 3 fasteners. Set the Collection tubes to the Collection tube holder (same number as sample number).

＜3＞ (Set of holders and waste containers) Set all the holder to each holder slot in 240L. Set the Waste container into the Waste container rack of the 240L drawer. Use the specific container and confirm that the Waste container is empty before use. The use of container not included in the delivery, use of un-emptied container or use with an erroneous setting may cause waste overflow.

＜4＞ (Set of lysate tube) Transfer the lysate prepared 7-3 into the specific lysate tube for QG-Auto240L. Open the Agitator cover and set the Lysate tubes with lysate on the Lysate unit in the 240L. After setting the Lysate tube, close the Agitator cover tightly.

＜5＞ (Isolation operation) Refer to the User's guide of QG-Auto240L "3.6 Start-up of System", turn on the power and proceed the mode select screen. Select "AUTOMATED OPERATION" and then select "W BLOOD DNA 2ml SEMI-AUTO". Press the numeric button corresponding to the number of set samples. Check that the selected button coincides with the number of set samples and press [OK]. Refer to the indicated information on the screen regarding the reagents to be used for the automatic isolating operation and confirm that the required quantity is set in the correct position. Press "CHECK" button of confirmed reagent and then press "OK" button. Automated operation is started when pressing "START" button.

＜6＞ (Finish operation/ Collect DNA) The operation ends when the "AUTOMATIC OPERATION END" is displayed. Turn the Power switch OFF. Open the Left flap door and take the Collection tube holder out. Close the Caps of the Collection tubes tightly. If you do not use DNA immediately, please close the tube lid tightly and store at 4°C or -20°C. In case storing cfDNA for a long time, preserve at -20°C.

＜7＞ (Clean up)
Remove Cartridge Holder and Waste tube Holder. Dispose the Cartridges, Waste tubes and waste fluid according to applicable regulations.
Refer to the User's guide of QG-Auto240L "3.9 Disposal of Consumables and Waste".

7-5.cfDNA isolation using QG-Mini8L

- Please read the User's Guide of QG-Mini8L for the details before using the system.
- Each accessories and consumables should be set at the correct position with following the order.
- Set the Waste tube, Cartridge and Collection tube at the correct position.
- Check that 160 ml of >99% ethanol is added to WCF before starting an experiment.
- Set Waste Tubes (WT) and Collection Tube (1.5 ml microtube) into the Tube Holder.
- Set the Waste Tubes and 1.5 ml microtubes into the Tube Holder in order. Then insert the Cartridge Holder into the correct position of the Tube Holder. Make sure the bulges of Cartridge Holder are inserted into the side notches of the Tube Holder. Set the Cartridges in the Cartridge Holder so that the position (No.) of each tubes is the corresponding position. Check the location of Waste tubes, 1.5 ml microtubes and Cartridges.
- Any solution and waste fluid containing LCF should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

Detailed isolation protocol with QG-Mini8L

<1> (Applying lysate) Carefully transfer the whole lysate prepared at 7-3 to each Cartridge. Make sure that the lysate does not touch the edge of the cartridge or around the cartridge. Set the Pressure Seal Plate covering the Cartridges. Then, set the Cartridge Holder and Tube Holder into QuickGene-Mini8L. Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine with a gentle push. After adding lysate and letting it stand for 10 minutes, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no liquid remains in the Cartridges and then return the Rotary Switch to the original position.
If any liquid is still remaining on the cartridge, add air pressure again after returning the Rotary Switch to the original position.

<2> (First Wash) Pull out the Cartridge Holder and the Tube Holder and then apply 7.5 ml of WCF to the Cartridges. If WCF adheres to the pressure seal plate, wipe it off with soft paper. Set the Pressure Seal Plate covering the Cartridges. Set the Cartridge Holder and Tube Holder into QG-Mini8L. Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine with a gentle push. Add the Wash Buffer and let stand for 5 minutes, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no WCF remains in the Cartridges and then return the Rotary Switch to the original position.

<3> (Second Wash) Pull out the Cartridge Holder and the Tube Holder and then apply 6.5 ml of WDT to the Cartridges. If WCF adheres to the pressure seal plate, wipe it off with soft paper. Set the Pressure Seal Plate covering the Cartridges. Set the Cartridge Holder and Tube Holder into QG-Mini8L. Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine with a gentle push. Add the Wash Buffer and let stand for 5 minutes, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no WCF remains in the Cartridges and then return the Rotary Switch to the original position.

<4> (Third Wash) Pull out the Cartridge Holder and the Tube Holder and then apply 5.5 ml of WCF to the Cartridges. If WCF adheres to the pressure seal plate, wipe it off with soft paper. Set the Pressure Seal Plate covering the Cartridges. Set the Cartridge Holder and Tube Holder into QG-Mini8L. Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine with a gentle push. Add

the Wash Buffer and let stand for 5 minutes, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Pressurize 5 times in this wash step to remove as much wash buffer as possible. Make sure that no WCF remains in the Cartridges and then return the Rotary Switch to the original position.

＜5＞ (Collection) Pull out the Cartridge/Tube Holder, Transfer the Cartridge Holder to the Elution position of the Tube Holder. Insert the Cartridge Holder into the side notches of the Tube Holder. Apply 100 µl of CCF to the Cartridges (CAL2) and set the Pressure Seal Plate covering the Cartridges (CAL2)Set the Cartridge Holder and Tube Holder into QG-Mini8L. Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine with a gentle push. After incubating at room temperature for 90 sec, Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no liquid remains in the Cartridges and then return the Rotary Switch to the original position. Refer to the User's guide of QG-Mini8L (p.22 2:Operation-collection)

＜6＞ (Finish and Clean up) Pull out the Cartridge Holder and the Tube Holder. Remove the Cartridge Holder from the Tube Holder, and then dispose of the Cartridges. Put Caps of the 1.5 ml microtubes and then collect them. Dispose of the Waste Tubes and waste solution according to appropriate laws and rules. If you do not use DNA immediately, please close the tube lid tightly and store at 4°C or -20°C. In case of storing cfDNA for a long time, it is recommended to preserve them at –20°C.

8. Trouble shooting

Review the information below to troubleshoot the experiments with QuickGene cfDNA isolation kit.

(1) Low yield or no DNA obtained

Cause	Possible Solution
Insufficient dissolution of protease (ECF).	Add nuclease-free ultrapure water, and vortex the bottle. Leave the solution 30-40 minutes and mix it a few times. Make sure if all the powder in the solution is dissolved completely before use.
Reagents and plasma added in the wrong order	Add the reagents and samples to 15 ml tube in the following order when preparing the lysate: Protease (ECF: dissolved in 3.3 ml of nuclease-free water) → plasma → Lysis Buffer (LCF).
Excess amount of samples was used.	Reduce the amount of plasma to below the specified amount.
Insufficient mixing at the addition of Lysis Buffer (LCF)	Mix sample immediately after Lysis Buffer (LCF) addition, shaking tube 10 times up and down and vortexing for 15 sec. with maximum speed. Recommending vortex speed is 2,500 rpm and more.
Requirement volume of ethanol was not added to Wash Buffer (WCF)	Always confirm that the required volume of ethanol was added to the Wash Buffer (WCF) prior to use.
Old wash buffer (WCF: including ethanol) used	Flash remaining Wash Buffer (WCF: including ethanol) which may be one day old or more in the instrument prior to use. Store the WCF with cap for long storage.

Insufficient mixing at the addition of Ethanol	Mix sample immediately after Ethanol addition, shaking tube 10 times up and down and vortexing for 15 sec. with maximum speed. Recommending vortex speed is 2,500 rpm and more.
Lysate is not fully applied to Cartridges (CAL2)	Insufficient vortexing, aggregates may be present in the lysate. Mix the sample thoroughly.
Insufficient amounts of reagents used.	Make sure that sufficient amount of reagent are in the reagent bottles.

(2) Clogging the cartridge

Cause	Possible Solution
Insufficient dissolution of protease (ECF).	Add nuclease-free ultrapure water, and vortex the bottle. Leave the solution 30-40 minutes and mix it a few times. Make sure if all the powder in the solution is dissolved completely before use.
Excess amount of sample was used.	Reduce the amount of plasma to below the specified amount.
Insufficient mixing at the addition of Lysis Buffer (LCF) or Ethanol	Mix sample immediately after Lysis Buffer (LCF) or Ethanol addition, shaking tube 10 times up and down and vortexing for 15 sec. with maximum speed. Recommending vortex speed is 2,500 rpm and more.

(3) Subsequent experiments (ex. PCR) unsuccessful

Cause	Possible Solution
Improper amount of DNA used for subsequent experiments	Determine the concentration based on the amount of cfDNA around 170bp by microchip electrophoresis.

(4) Supplying the precipitates in reagents

Cause	Possible Solution
Stored at low temperature	Store solutions at 15-28°C. If the precipitates are contained, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved.

9. Ordering Information

Product	Cat #
QuickGene DNA whole blood kit L	DB-L
QuickGene DNA tissue kit L	DT-L
QuicGene cfDNA isolation kit	CF-L
QuickGene Auto240L Consumables Kit	240L-CK

Appendix. Preparetion of plasma

We introduce a preparation method carrying out as an example in our comany.

1. Collect whole blood into a blood collection tube or a tube of appropriate size.
   For example, collect 10 ml of whole blood into a 15 ml centrifuge tube, etc.
2. Centrifuge at 1,900 × g for 15minutes at room temperature.
3. Carefully aspirate the plasma supernatant without disturbing the separating layer.
   About 4-5 ml of plasma can be collected from 10 ml of whole blood.
4. Centrifuge at 1,900 × g for 15minutes at room temperature.
5. Carefully aspirate the plasma supernatant and transfer to a new tube.
6. The collected plasma will be used for cfDNA separation.
   In the case QG-Auto240L-Automated protocol, collect plasma in a sample tube of the appropriate blood collection tube size described in "p.5 3-[4] Additional required materials when using QG-Auto240L ".

* Trademark and exclusion item

Right to registered name etc. used in this handbook is protected by law especially even in the case of no denotation.

KURABO INDUSTRIES LTD.
Bio-Medical Department, Advanced Technology Division
14-30, Shimokida-Cho, Neyagawa, Osaka 572-0823, Japan
TEL; +81-72-820-3079 FAX; +81-72-820-3095
URL; https://www.kurabo.co.jp/bio/English

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