KURABO

HANDBOOK

QuickGene DNA whole blood kit L (DB-L)

For Isolation of Genomic DNA from whole blood

Ver.2.3

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 genomic DNA 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.

○DNA from whole blood samples can be simultaneously extracted in following time.

・QuickGene-Auto240L (QG-Auto240L):about 60 min for 24 sets of whole blood sample

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

・QuickGene-Mini8L (QG-Mini8L):about 20 min for 8 sample

・QuickGene-610L (QG-610L):about 12 min for 6 sample

2. Kit components and storage conditions

2-1. Kit components

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

Name	Number	Volume
Protease (EDB)	5 vials	Lyophilized
Lysis Buffer (LDB)	2 bottles	70 ml
Wash Buffer (WDB)	4 bottles	160 ml
Elution Buffer (CDB)	1 bottle	100 ml
Cartridges (CAL2)	1 bag	48 pcs
Waste Tubes (WTL)	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 (EDB) will be able to store for two months at 4°C.
Storage at -20°C will prolong the life of EDB, 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, QG-Mini8L or QG-610L

- 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 CDB container.

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

[3] Additional required materials when using QG-Mini8L and QG-610L

- 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)

(***) : Use 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 EDB

- 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.

◆ LDB (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.

◆ WDB (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.

◆ CDB (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

Precautions

- Corresponding sample: whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Use a whole blood sample within 3 days after collection.
- Small amount of samples should be adjusted to 2 ml with PBS (sterilized) before loading.
- The yield of DNA might decrease when the number of leucocytes exceeds 2×10^7 cells. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^7 cells.
- The Cartridge (CAL2) might clog when the number of leucocytes exceeds 5×10^7 cells. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.
- Use the whole blood sample as much early as possible. Extract DNA from the whole blood sample stored at 4°C in 3 days after collection.
- 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 (LDB) from heat (more than 28°C). Do not mix with disinfectants such as bleach.
- Default elution volume is 500 µl. In case of setting to less than 500 µl, yield may decline.
- In case of using QG-610L, directly set Wash Buffer (WDB) bottle including dedicated volume of ethanol and Elution Buffer (CDB) in centrifuge tube at the correct position. Make sure to complete discharge at the beginning of use. Refer to the User's guide of QG-610L.

6. Quality controls

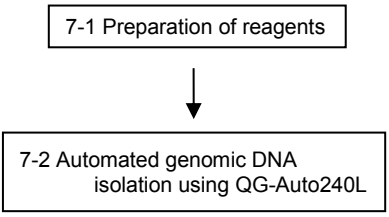
As part of the stringent of quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene DNA whole blood kit L is evaluated routinely.

Yield and quality of extracted DNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

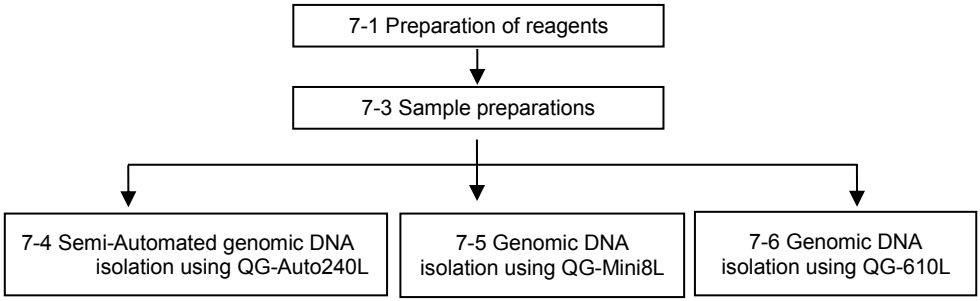
7. Protocols

【Overview Flow Chart】

① –In case of QG-Auto240L-Automated-



② –In case of QG-Auto240L-Semi-Automated-, QG-Mini8L or QG-610L-



7-1. Preparation of reagents

◆Protease (EDB)

When using EDB, pipette 3.3 ml of nuclease-free water into the vial containing lyophilized Protease. Dissolve it completely. Reconstituted EDB is stable for 2 months when stored at 4°C. Storage at -20°C will prolong the life of EDB 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 EDB 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 EDB is insufficient.

◆Lysis Buffer (LDB)

Mix thoroughly before using. If the precipitates are contained in Lysis Buffer (LDB), 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 (LDB), cool down the bottle to room temperature before using.

◆Elution Buffer (CDB)
Make sure to use CDB for DNA elution.

◆Wash Buffer (WDB)
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. A bottle of WDB is available for 12 samples preparation.

Requirements of Wash Buffer (WDB) with >99% ethanol and Elution Buffer (CDB)
Prepare the requirements of Wash Buffer (WDB) with >99% ethanol and Elution Buffer (CDB) according to the number of samples for isolation; refer to the following table. Set the bottle on the QuickGene-610L. (See the user's guide of QuickGene-610L.)
Put appropriate amount of CDB into 50 ml centrifuge tube and set the tubes in the QuickGene-610L tube holder. (See the user's guide of QuickGene-610L.)

Number of samples	WDB with Ethanol	CDB
6	160 ml (1/2 bottles)	11 ml
12	320 ml (1 bottle)	16 ml
18	480 ml (1 1/2 bottles)	24 ml
24	640 ml (2 bottles)	32 ml
30	800 ml (2 1/2 bottles)	40 ml
36	960 ml (3 bottles)	48 ml
42	1120 ml (3 1/2 bottles)	56 ml
48	1280 ml (4 bottles)	64 ml

7-2. Automated genomic DNA 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) Mix the sample of vacutainer by inverting gently. Remove the lid of vacutainer. Set the Sample holder to the Sample holder slot in 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 "W BLOOD 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 genomic DNA for a long time, preserve at -20°C.

7-3. Sample preparations

- The QuickGene DNA whole blood kit L is specifically designed for genomic DNA isolation from 2 ml of whole blood. Recommend using the whole blood collected in EDTA·2Na, EDTA·2K or heparin.
- Please keep the below sample volume. Excess amount of sample may occur low isolation efficiency or interference with device operation.

1. Follow the protocol of 1a to 1c exactly. If you change the protocol, may be reduced the yield. You can use 50ml centrifuge tube instead of 15ml tube.
   - <1a> 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 EDB to bottom of 15 ml tube.
   - <1b> Add 2 ml of whole blood into the 15 ml tube, and then add 2.5 ml of LDB immediately. (Leaving the samples long time before addition of LDB may be reduced the yield.)
   - <1c> Mix the sample and LDB with shaking 10 times up and down.

It is very important to mix thoroughly the sample after addition of LDB.

2. 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.

3. 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.

4. Add 2.5 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 genomic DNA 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 genomic DNA for a long time, preserve at -20°C.

7-5. Genomic DNA isolation using QG-Mini8L

- Please read the User's Guide of QuickGene-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 WDB before starting an experiment.
- Set Waste Tubes (WTL) 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 LDB 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. 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 WDB to the Cartridges. If WDB 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 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. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no WDB 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 WDB to the Cartridges. If WDB 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 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. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no WDB 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 WDB to the Cartridges. If WDB 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 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. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no WDB 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 500 µl of CDB to the Cartridges (CAL2) and set the Pressure Seal Plate covering the Cartridges (CAL2). 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. 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> 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 genomic DNA for a long time, it is recommended to preserve them at -20°C.

7-6. Genomic DNA isolation using QG-610L

- Please read the User's Guide of QuickGene-610L for the details before using the system.
- Each accessories and consumables should be set at the correct position with following the order.
- Set Waste tube, Cartridge and Collection tube at collect position.
- Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.
- Make sure to discharge after setting of Wash Buffer (WDB) and Elution Buffer (CDB).
- Any solution and waste fluid containing LDB 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-610L

＜1＞ (Selection of Isolation mode)Select “DNA WHOLE BLOOD” mode for genomic DNA isolation from whole blood with the kit. (See Appendix 1)

＜2＞ (Setting of consumables) Open the front cover of the instrument and set the collection tube (1.5 ml micro tube) in the Tube Holder and Waste Tube (WTL) into Holder Carriage.

＜3＞ (Setting of reagents） Prepare the required volume of Wash Buffer (WDB) with >99% ethanol and Elution Buffer (CDB) into the tubes; set them to the holder; and put the holder to the designated positions of instrument.

- The required reagent volume is different from number of samples.
- Refer the user’s guide for the QuickGene-610L for details for setting reagents.

＜4＞ (Discharge) Set the “Discharge Tray” and check the Tube Holder and Cartridge Holder setting for the correct positions. Press the [DISCHARGE] after closed the front cover of the instrument.

- Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

＜5＞ (Applying the lysate) Apply all contents of prepared lysate samples (see 7-3 Sample preparations) into the each Cartridge (CAL2) decantation or using micropipettes (any aggregates in the lysate should be transferred into the cartridge). Please note that do not put lysate on the edge of Cartridge. Put the cap of the Cartridge Holder onto Cartridge and rock it with two ratchets. Set the Cartridge Holder onto the Holder Carriage.

＜6＞ (Isolation) Close the front cover of the instrument. Confirm the appropriate mode on the operation panel and press the [START] button. After starting of isolation,【EXECUTING】is displayed on operation panel. Do not open the front cover during isolation operation. If it is opened, operation is forced terminated.

＜7＞ (Collection) When the sound is heard, the separation is complete.
After completing the process, each sample result is indicated on the operation panel as follow;

V (Check)	Completed normally
- (Hyphen)	Not completed normally
_(Underscore)	No cartridge or no sample

After confirming that the device is completely stopped, open the front cover, take out the 1.5 ml microtube from the Collection holder. Cover with the caps on the collection tube containing the isolated genomic DNA and store it at the appropriate condition. In case of storing genomic DNA 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 DNA whole blood kit L.

(1) Low yield or no DNA obtained

Cause	Possible Solution
Insufficient dissolution of protease (EDB).	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 whole blood added in the wrong order	Add the reagents and samples to 15 ml tube in the following order when preparing the lysate: Protease (EDB: dissolved in 3.3 ml of nuclease-free water) → whole blood → Lysis Buffer (LDB).
Excess amount of samples was used.	Reduce the amount of whole blood to below the specified amount.
Excess amount of leukocyte	A sample contained over 2×10^7 of leukocyte cells, the yield may decrease. In the case of sample, dilute the sample not over 2×10^7 by PBS.
Insufficient mixing at the addition of Lysis Buffer (LDB)	Mix sample immediately after Lysis Buffer (LDB) 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 (WDB)	Always confirm that the required volume of ethanol was added to the Wash Buffer (WDB) prior to use.
Old wash buffer (WDB: including ethanol) used	Flash remaining Wash Buffer (WDB: including ethanol) which may be one day old or more in the instrument prior to use. Store the WDB 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 (EDB).	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 whole blood to below the specified amount.
Excess amount of leukocyte cells	A sample contained over 2×10^7 of leukocyte cells, the yield may decrease. In the case of sample, dilute the sample not over 2×10^7 by PBS.
Insufficient mixing at the addition of Lysis Buffer (LDB） or Ethanol	Mix sample immediately after Lysis Buffer (LDB) 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 absorbance at 260 nm.

(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.

(5) The collection tubes are empty after the elution

Cause	Possible Solution
Missed the discharge	According to 7-6-<4>, discharge should be operated after setting of Wash Buffer (WDB), Elution Buffer (CDB). (Refer to the operation manual of QG-610L for details).

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

● Appendix1 Parameter of QG-610L

"DNA WHOLE BLOOD" mode of QG-610L use the below parameter table. It should be used for this kit. Please refer to the User's guide of QG-610L to know how to change the parameter.

	DNA WHOLE BLOOD	
No.	PARAMETER	SET VALUE
1	BIND PEAK	120
2	WASH COUNT	3
3	WASH PEAK	90
4	WASH VOL1	7500
5	WASH VOL2	6500
6	WASH VOL3	5500
7	WASH VOL4	0
8	WASH VOL5	0
9	WAS2 COUNT	0
10	WAS2 PEAK	90
11	WAS2 VOL1	7500
12	WAS2 VOL2	6500
13	WAS2 VOL3	5500
14	WAS2 VOL4	0
15	WAS2 VOL5	0
16	ELUT VOL	500
17	ELUT PEAK	100

* 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.

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DB-L_HB-E_V23