KURABO

HANDBOOK

QuickGene DNA whole blood kit L (DB-L)

For Isolation of Genomic DNA from whole blood

Ver.2.0

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 isolates 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.
When QuickGene DNA whole blood kit L is used with Automatic Nucleic Acid Isolation Systems (QuickGene-610L), high quality and high yield genomic DNA can be isolated and also purified from whole blood. In addition, DNA from 6 sets of whole blood samples can be simultaneously extracted in only 12 minutes. The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, southern blotting and other applications.

Please be sure to read this handbook carefully before using the kit.
This Kit is only used with QuickGene-610L.

2. Kit components

The kit includes the reagents necessary for 48 sets of genomic DNA isolation.

□ Protease	(EDB)	5 tubes
□ Lysis Buffer	(LDB)	2 bottles
□ Wash Buffer	(WDB)	4 bottles
□ Elution Buffer	(CDB)	1 bottle
□ Cartridges	(CAL2)	
□ Waste Tubes	(WTL)	

3. Storage conditions

All reagents are stable for one year at room temperature (15-28°C). The dissolved protease (EDB) will be able to store for two months at 4°C.

4. Other required materials, not supplied in this kit

◆ **Reagents**
- >99% Ethanol
- Nuclease-free ultra pure water (for dissolving proteases)

◆ **Instruments and equipments**
- QuickGene-610L
- 50 ml and 15 ml centrifuge tubes*
- Micropipettes and tips
- 1.5 ml micro tubes (for elution collection)**
- Vortex mixer
- Tube stands
- Table top water bath (for incubation of 50 ml or 15 ml centrifuge tubes at 56°C)
- 500 ml reagent bottle (for keeping the caps of wash buffer bottle)

*; 15ml (or 50ml) centrifuge tubes are used for sample preparation. 50 ml centrifuge tube is used for the containers for Elution Buffer (CDB) for QuickGene-610L.
Recommended centrifuge tube; BD Falcon™ 50 ml, 15 ml conical tube.

**; Recommended micro-centrifuge tube; Eppendorf™ 1.5 ml Micro Standard tube.

Table1 Recommended centrifuge tubes

Type of centrifuge tube	Product name (Examples)
50 ml centrifuge tube	BD Falcon™ 50 ml conical tube
15 ml centrifuge tube	BD Falcon™ 15 ml conical tube

5. Safety warnings

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

- All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, gloves and safety glasses 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 Material Safety Data Sheet for specific recommendations, http://www.kurabo.co.jp/bio/English/)

Protease (EDB)
Do not put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Lysis Buffer (LDB)
Poisonous if swallowed
Do not put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.
Wear laboratory coat, gloves and safety glasses during experiments.

Wash Buffer (WDB)
Do not put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Elution Buffer (CDB)
Do not put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

- Keep away the Lysis Buffer (LDB) from heat. Do not mix with disinfectants such as bleach.
- For disposal of waste fluid and consumables: When using potentially infectious samples for experiments, dispose them according to applicable regulations.

6. Precautions

- Refer to the MSDS (Material Safety Data Sheet) for specific recommendations on properties and handling. The MSDS can be obtained from the World Wide Website (http://www.kurabo.co.jp/bio/English/).
- Refer to the user's guide for the QuickGene-610L before using.

7. Quality controls

- The stability of the reagents is guaranteed for one year after purchase if stored at the specified temperature (15-28°C).
- 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 on a lot-to-lot uniformity.
- Quality and yield of isolated genomic DNAs are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

8. Protocols

8-1 Preparation of reagents

Protease (EDB)

Add 3.3 ml of nuclease-free ultra pure water to the vial containing the freeze-dried protease, and dissolve it carefully.
Store the dissolved protease (EDB) 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 -20°C. Recommend to avoid repeated freezing and thawing.

Notice: Use the protease (EDB) after dissolving it completely with the following instructions.
Add 3.3ml of nuclease-free ultra pure water, and vortex with the cap closed.
Leave the protease (EDB) solution 30-40 minutes in room temperature and mix it a few times.
Make sure if all the powder in the solution is dissolved completely before use.
If it is not dissolved completely, the yield would be insufficient or the cartridges would be clogged.

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.

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 50ml centrifuge tube and set the tubes in the QuickGene-610L tube holder. (See the user's guide of QuickGene-610L.)

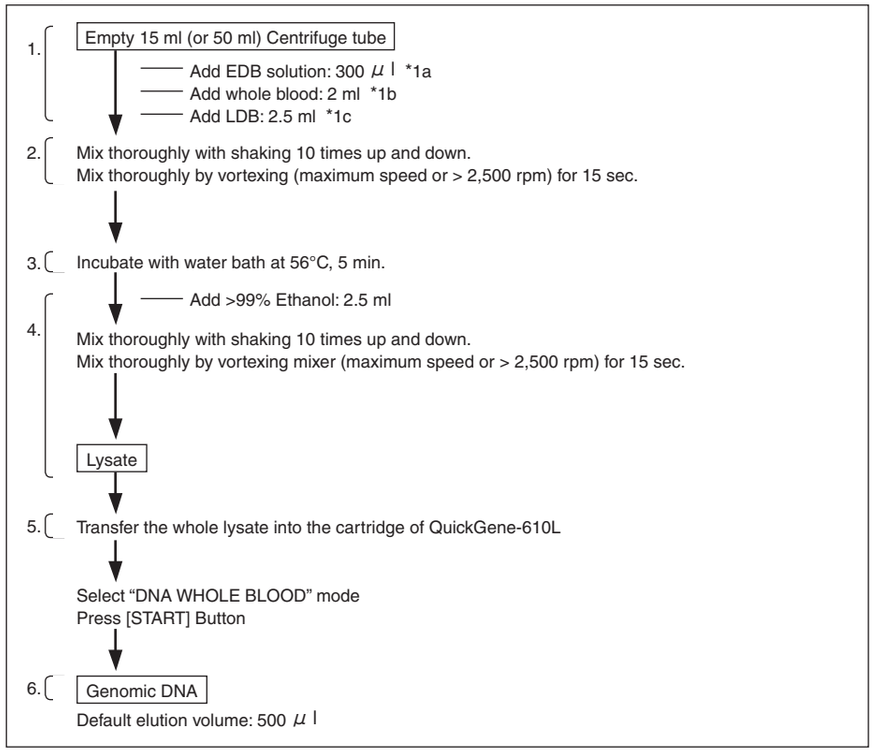
Table2 Buffer volume and the number of samples to set in the QuickGene-610L

Number of samples	WDB with Ethanol	CDB
6	160 ml (1/2 bottle)	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

8-2 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.
- The yield will depend on the sample condition.
- Use the kit at room temperature (15-30°C). When using the kit at lower or higher temperatures, the expected yield may not be obtained.
- Accurately measure the buffer volume during the experiments.

<Preparation workflow from whole blood>



Notice

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 ultra pure 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 of QuickGene-610L, or low yield.
3. Incubate with water bath at 56°C 5 min. The maximum incubation time is 10 min.
   When you use 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.
   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 of QuickGene-610L, or low yield.
5. Transfer the whole lysate to the cartridge of QuickGene-610L. Perform isolation within 30 min. after lysate preparation.
   If aggregates are present in the lysate, apply them along with the lysate to the cartridge.
6. Default elution volume is 500 μl. In case of setting to less than 500 μl, yield may decline.
   The standard yield of eluted genomic DNA is 30-80 μg from 2 ml whole blood.
   Store the eluted genomic DNA at -20°C for long storage.
   Two times elution program can increase the yield of DNA for 10-20% with another 500 μl Elution Buffer (CDB) (total Elution Buffer (CDB) volume is to be 1 ml). Please refer to 8-3 and the user’s guide of QuickGene-610L for setting the program.

8-3 Genomic DNA isolation using the QuickGene-610L

Notice: System set up and basic operations.
Please read the user's guide of QuickGene-610L circumstantially for the details before using the system.

(1) Selection of isolation mode

Select "DNA WHOLE BLOOD" mode for genomic DNA isolation from whole blood with the kit. (See Appendix 1)

Setting for the two times elution program: Change the parameter of "ELUT COUNT" in the "EXPERT" mode from "1" to "2". Please refer to the user's guide of QuickGene-610L for changing the parameters.

Notice: Incorrect parameters in "EXPERT" mode may damage the instrument and waste samples.

(2) Setting of cartridges and tubes

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.

- Use the 1.5 ml micro tube for elution and Waste Tube (WTL) including the kit for waste.
- Use the specified Cartridges (CAL2).

Notice: Refer to the user's guide for the QuickGene-610L for details of setting cartridges, tubes and bottles.
Incorrect cartridge placement may result in the solution spilling or improper isolation.
Wear gloves during the experiments to avoid nuclease contamination.

(3) Setting of reagents

Prepare the required volume (see 8-1 Preparation of reagents) 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.

Notice: Wear gloves during the handling of reagents to avoid nuclease contamination.
- 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.

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

(5) Applying the prepared samples

Apply all contents of prepared lysate samples (see 8-2 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

Check if the materials—Wash Buffer (WDB) with >99% ethanol, Elution Buffer (CDB), Cartridges (CAL2) including samples, Waste Tubes (WTL), and collection tubes are well setting.

Close the front cover of the instrument.

Confirm the appropriate mode on the operation panel and press the [START] button.

(7) Collection of genomic DNA

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

Open the front cover and remove the collection tube(s) from the Tube Holder.

- As genomic DNA is eluted from the Cartridge(s) (CAL2) using 500 μ l of Elution Buffer (CDB), the volume of recovered total DNA solution will be 500 μ l.

Cover with the caps on the collection tube containing the isolated genomic DNA.

(8) Clean up

Remove the Waste Tubes (WTL) and dispose the waste fluid according to applicable regulations.

Remove the Cartridge Holder and dispose the Cartridges (CAL2).

Warning: Disposal of waste fluid and consumables.
When using the potentially infectious samples for experiments, dispose them according to applicable regulations.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene DNA whole blood kit L. For system-related problems (e.g., when an error message appears), see the QuickGene-610L user's guide.

(1) Low yield or no DNA obtained

Cause	Possible Solution
Insufficient dissolution of protease (EDB).	Add nuclease-free ultra pure 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 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)	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 Cartridge(s) (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 ultra pure 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 (e.g., 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	Set the "Discharge Tray" and check the Tube Holder and Cartridge Holder setting up into correct positions. Press the [DISCHARGE] after closed the front cover of the instrument. See the QuickGene-610L user's guide.

10. Ordering Information

Product	Cat #
QuickGene-610L Automatic Nucleic Acid Isolation Systems	
QuickGene DNA whole blood kit L	DB-L
Dedicated reagent kit for QuickGene-610L to isolate the Genomic DNA from whole blood	

Appendix 1 "DNA WHOLE BLOOD" mode is set in the following parameter.

	DNA WHOLE BLOOD
PARAMETER	SET VALUE
BIND PEAK	120
WASH COUNT	3
WASH PEAK	90
WASH VOL1	7500
WASH VOL2	6500
WASH VOL3	5500
WASH VOL4	0
WASH VOL5	0
WAS2 COUNT	0
WAS2 PEAK	90
WAS2 VOL1	7500
WAS2 VOL2	6500
WAS2 VOL3	5500
WAS2 VOL4	0
WAS2 VOL5	0
ELUT VOL	500
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.

KURABO

KURABO INDUSTRIES LTD.

Bio-Medical Department
Kurabo Neyagawa Techno Center 3F, 14-5, Shimokida-Cho, Neyagawa,
Osaka 572-0823, Japan
TEL +81-72-820-3079　FAX +81-72-820-3095
URL; http://www.kurabo.co.jp/bio/English/

DB-L_HB-E_V20