KURABO

HANDBOOK

QuickGene DNA whole blood kit S (DB-S)

For extraction of genomic DNA from whole blood

Ver.3.0

Contents

Warning For research use only.
Not recommended or intended for diagnostic or clinical application for humans or 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.

When using this kit with QuickGene, high quality and high yield genomic DNA can be extracted and also purified from whole blood. No hazardous organic solvents such as phenol and chloroform are used. DNA from 8 sets of whole blood lysate samples can be simultaneously extracted in following time.
QuickGene-810/QuickGene-800 (QG-810/QG-800) : about 6 min
QuickGene-Mini80 (QG-Mini80) : about 6 min
The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, Southern blotting and other applications.

Please be sure to read the User's Guide of QuickGene carefully before using this kit.

2. Kit Components and Storage Conditions

2-1 Kit Components (96 Preps)

☐ Protease	EDB	1 vial
☐ Lysis Buffer	LDB	30 ml
☐ Wash Buffer	WDB	160 ml
☐ Elution Buffer	CDB	100 ml
☐ Cartridges	CA	96
☐ Collection Tubes	CT	96
☐ Caps	CAP	96
☐ Waste Tubes	WT	96

2-2 Storage Conditions

All reagents are stable for one year after purchase at room temperature (15-28°C). Reconstituted EDB is stable for 2 months when stored 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] Reagents
- 99% Ethanol (for preparation of lysate and WDB working solution)
- Nuclease-free water (for dissolving EDB)

[2] Equipments
- QuickGene
- Centrifuge tubes * (Large/Small sets)
- Micropipettes and tips
- 1.5 ml microtubes
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Benchtop microcentrifuge
- Heat block or water bath (at 56°C)

*Centrifuge tubes are used with QG-810/QG-800 as containers for WDB (>99% ethanol added) and CDB. They are unnecessary when QG-Mini80 is used.

Recommendation product of centrifuge tubes are following Table 1.
Use centrifuge tubes according to the number of Cartridges to use.

Table 1 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WDB)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CDB)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WDB)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CDB)	BD Falcon™ 50 ml conical tube

4. Safety Warnings

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- 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 Material Safety Data Sheet for specific recommendations, http://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.

◆ Use or storage of LDB at high temperature should be avoided.

◆ Any solution and waste fluid containing LDB should not be mixed with bleach.

◆ **In the case of using potentially infectious samples :**
Wear a suitable laboratory coat, disposable gloves and safety goggles during the experiments.

◆ **Disposal of waste fluid and consumables when using potentially infectious samples :**
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

◆ Handling of Starting Material
- Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
- Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Use a whole blood sample within 3 days after collection. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
- The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6 cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
  The Cartridge (CA) might clog when the number of leucocytes exceeds 5×10^6 cells/200 µl. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.

◆ Use of Reagent
- After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CA) might clog when dissolution of EDB is insufficient.

◆ Procedure of Extraction
- Use QuickGene DNA whole blood kit S (DB-S) at room temperature (15-30°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Use a vortex mixer able to stir at 2,500 rpm or more. Weak vortex may cause insufficient dissolution, lead to decrease of the yield of DNA or clogging of the Cartridge (CA).
- During the procedure, work quickly without interruption.
- The yield of DNA varies depending upon sample conditions. The standard yield is 4 to 8 µg from 200 µl whole blood samples.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : 8-3 (p.14), Appendix 1 (p.26), Appendix 2 (p.27)
  QG-Mini80 : 8-4 (p.17)
- Refer to QuickGene User's Guide for details.

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene DNA whole blood kit S (DB-S) is evaluated routinely on a lot-to-lot uniformity.
- Yield and quality of extracted genomic DNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

7. Product Description

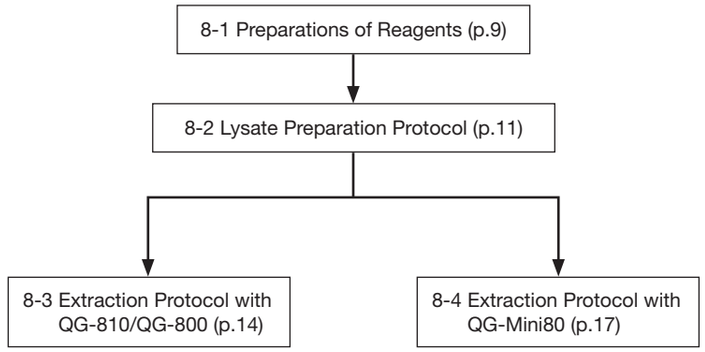
DNA and RNA are included in eluate extracted with this kit. Table 2 shows the average of yield and purity (A260/280) of genomic DNA extracted from 200 µl of whole blood samples.
The yield varies depending upon sample conditions.

Table 2

Sample	Amount of genomic DNA (µg)	A260/280
Whole blood (200 µl)	4-8	1.97

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

◆ EDB (Lyophilized)

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, 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 (CA) might clog when dissolution of EDB is insufficient.

◆ LDB (30 ml)

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.

◆ WDB (160 ml)

WDB is supplied as a concentrate.
Add 160 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ CDB (100 ml)

Use CDB for elution of genomic DNA.

◆ Required volume of WDB (>99% ethanol added) and CDB (In the case of using QG-810/QG-800)

Prepare the required volume of WDB and CDB into the tubes (see Table 1 p.5) : set them to Buffer Stand.

Table 3 Required volume of WDB and CDB

Number of Cartridges	WDB (QG-810/QG-800)	CDB (QG-810)	CDB (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WDB 8.0 ml, CDB 7.4 ml
QG-800 : WDB 8.0 ml, CDB 6.4 ml
Depending on the number of the Cartridges, add WDB and CDB.
Use WDB 2.25 ml and CDB 200 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WDB, 7.8 ml of CDB (QG-810) and 6.8 ml of CDB (QG-800) are required.
*Use appropriate tubes according to Table 1 (p.5).

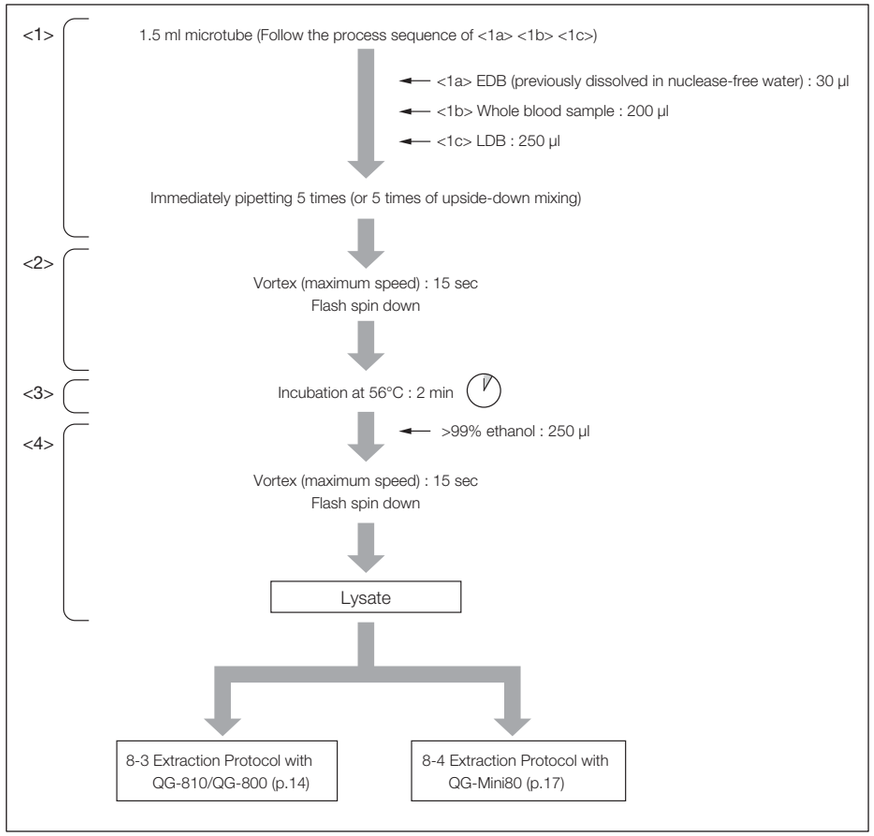
8-2 Lysate Preparation Protocol

QuickGene DNA whole blood kit S (DB-S) corresponds to the extraction of genomic DNA from 200 µl of whole blood sample per each treatment.

[Important notes before starting]
- Cool down all reagents to room temperature before use.
- Set the temperature of a heat block or a water bath to 56°C (it is used in step <3> p.13).
- Follow the volume of samples and buffers described in the workflow (p.12).
- During the procedure, work quickly without interruption.
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- Any solution and waste fluid containing LDB should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

[Preparations for starting the experiment]
- WDB is supplied as a concentrate. Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.

Lysate Preparation Workflow



Details of Lysate Preparation Workflow

<1> Follow the protocol of <1a> to <1c> exactly. Do not add LDB directly after addition of EDB to a 1.5 ml microtube. In case the procedure is changed, the yield of DNA may not be obtained.

<1a> Add 30 µl of EDB (previously dissolved in nuclease-free water) to the bottom of a 1.5 ml microtube.

<1b> Add 200 µl of a whole blood sample.

After adding the whole blood, immediately proceed to step <1c>.
Leaving the samples for a long time before addition of LDB might decrease the yield of DNA.

<1c> Add 250 µl of LDB, then immediately pipette 5 times.

Instead of pipetting, mixing upside-down 5 times can be performed.
In order to ensure efficient lysis, it is essential to mix throughly the sample and LDB. Pipette (or mix upside-down) surely in order to mix EDB, whole blood sample and LDB efficiently.

<2> Vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

Surely vortex for 15 sec at the maximum speed. The speed of 2,500 rpm or more is recommended. If you do not have such a vortex mixer, pipette (or mix upside-down) completely at step <1c>.
In case mixing is insufficient, the yield of DNA might decrease or the Cartridge (CA) might clog.

<3> Incubate at 56°C for 2 min.

Prolongation of the incubation time up to 5 min does not affect the yield.

<4> Add 250 µl of >99% ethanol, and vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

Mix the sample and the ethanol enough. Vortex at the same speed as in step <2>.

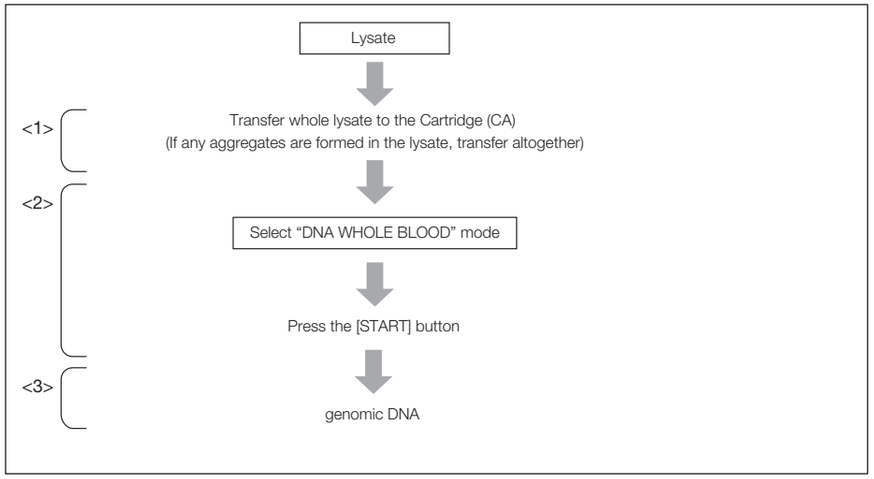
Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.14)
QG-Mini80 (p.17)

8-3 Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.
- Select "DNA WHOLE BLOOD" mode as the extraction mode for QG-810/QG-800.
- All reagents, Cartridges (CA) and tubes are manufactured in clean rooms. Wear gloves during the experiments to avoid nuclease contamination.
- Refer to the User's Guide of QG-810/QG-800 for the details of setting Cartridges (CA), tubes and each reagent.
- Open the front cover of QG-810/QG-800 and set the Collection Tubes (CT) and the Waste Tubes (WT) in the Tube Holder (or Collection Tube Holder). Use the specified Cartridges (CA).
- Set WDB (>99% ethanol added) and CDB to QG-810/QG-800 referring to p.10.
- Incorrect Cartridge (CA) placement may result in the solution spilling or improper extraction.
- Press the [DISCHARGE] button after closed the front cover of QG-810/QG-800. Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- Any solution and waste fluid containing LDB should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

QG-810/QG-800 Workflow



Details of QG-810/QG-800 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate (See section 8-2 p.11) to the each Cartridge (CA).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Perform the extraction operation quickly after completion of lysate. It is possible to leave it until 30 min if necessary.

<2> <Extraction> Select the appropriate mode for this kit. In case of confirming the setting of parameter, refer to Appendix 1 (p.26), Appendix 2 (p.27). Close the front cover of QG-810/QG-800. Confirm the appropriate mode on the operation panel and press the [START] button.

The operation panel shows "PROCESSING" (QG-810) or "EXECUTING" (QG-800) during extracting. In case of using QG-810, extraction progress can be checked by blinking of each lamp (BINDING, WASHING, ELUTION).

Warning Do not open the front cover during the extraction process (while "PROCESSING" or "EXECUTING" is shown on the operation panel). If the front cover is opened, the extraction process will be halted. Confirm it by Table 4.

Table 4 Movement when you opened a front cover during extraction

	QG-810	QG-800
Extraction process	Stop	Stop
Extraction continuation	Possible*1	Impossible*2

*1 QG-810 : See User's Guide of QG-810, "3.5 Operations to Restart Program from Pause" (p.28).
*2 QG-800 : The sample that was on the way of the extraction cannot be used again. Discard according to the applicable regulations. Refer to "Disposal of waste fluid and consumables when using potentially infectious samples" of this handbook (p.6).

<3> <Extraction completion>
Operation panel displays the extraction results.

Table 5 Extraction result

	QG-810	QG-800	Remarks
Successfully extracted	v (Check)	○	
Extraction failure	- (Hyphen)	×	Cartridge is clogged
No Cartridge, or No sample	_ (Underscore)	▲	No Cartridge or occurrence of error before extraction

Open the front cover and remove the Collection Tubes (CT) from the Tube Holder after QG-810/QG-800 completely stopped.
The volume of the eluate from each Cartridge will be 200 µl.

The volume of CDB can be reduced to 50 µl, but in that case, elution efficiency might decrease by about 20%.
Refer to Appendix 1 (p.26 for QG-810) or Appendix 2 (p.27 for QG-800) to change the volume of CDB.
The standard yield is 4 to 8 µg from 200 µl of whole blood samples.
Cover with the Caps (CAP) on the Collection Tubes (CT) tightly, store at 4°C or –20°C.
In case of storing genomic DNA for a long time, it is recommended to preserve at –20°C.

<4> Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.
Remove the Cartridge Holder and dispose the Cartridges (CA).
Dispose the fluid in the Discharge Tray also.

8-4 Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 160 ml of >99% ethanol is added to WDB before starting an experiment.
- Set Waste Tubes (WT) into the Tube Holder.
- Set Tube Adapters to the Tube Holder, and set Collection Tubes (CT). In substitution for Collection Tubes, you can use 1.5 ml microtubes. In this case Tube Adapters are unnecessary.
- Insert the Cartridge Holder into the Wash Position (W) of the Tube Holder. Then set Cartridges (CA) to the Cartridge Holder. Confirm the Release Lever is positioned at the left-hand end of the Cartridge Holder.

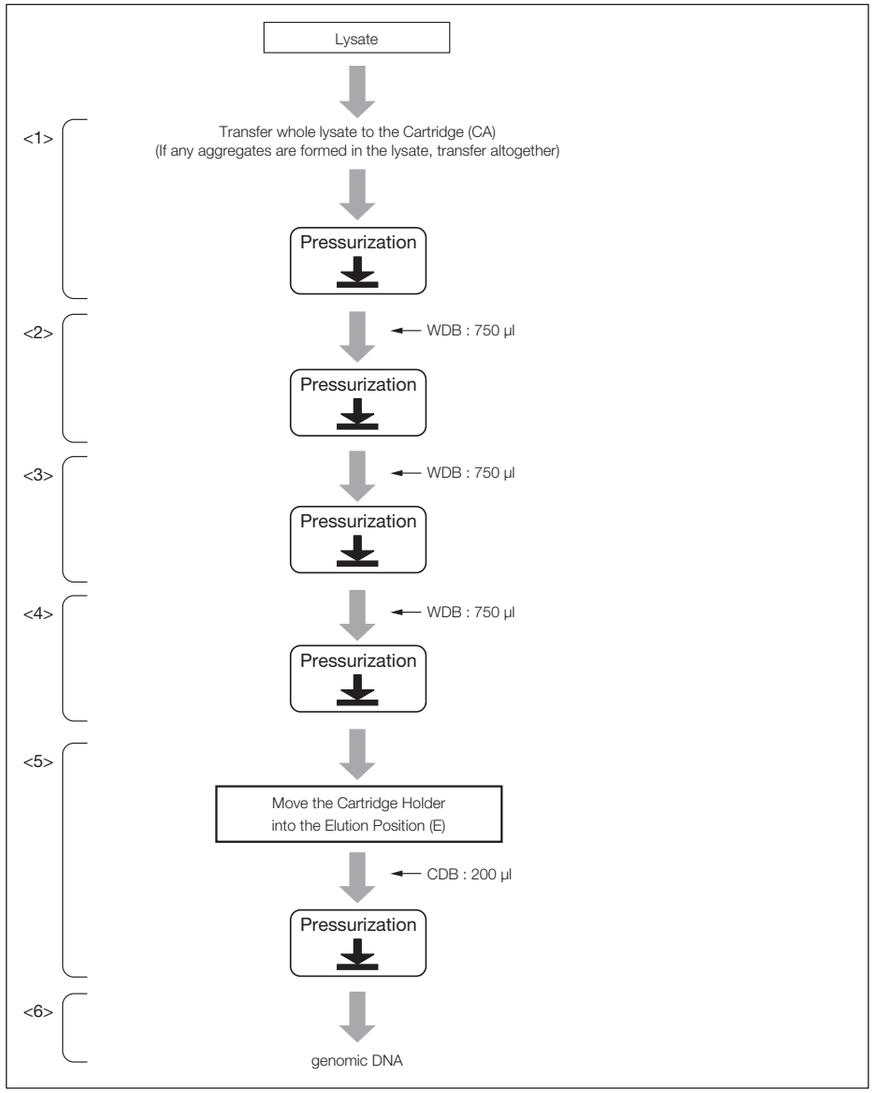
- When setting the Cartridge Holder and the Tube Holder to QG-Mini80, insert to the end.
- When pressuring lysates and WDB (>99% ethanol added), confirm that the Wash Label on the Tray can be entirely seen.
- When pressuring CDB, confirm that the Wash Label on the Tray can not be seen, being hidden below the Tube Holder.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- 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.

QG-Mini80 Workflow

The pressurization mark “Pressurization” in the workflow indicates the following operations.

1. Set the Cartridge Holder and the Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.



Details of QG-Mini80 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate prepared at 8-2 (p.11) to each Cartridge (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no lysate remains in the Cartridges and then return the Rotary Switch to the original position.

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Perform the extraction operation quickly after completion of lysis. It is possible to leave it until 30 min if necessary.
Pressure application automatically stops in about 1 min. If any lysate remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

<2> <First wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDB to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. 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.

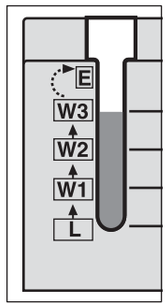
Pressure application automatically stops in about 1 min. If any WDB remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

<3> <Second wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDB to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. 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.

Pressure application automatically stops in about 1 min. If any WDB remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

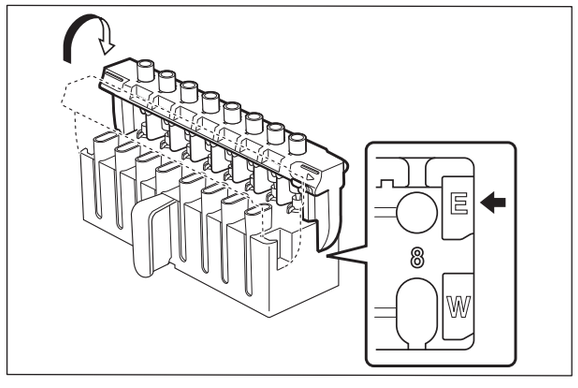
<4> <Third wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDB to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WDB remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WDB remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
After third wash, the waste fluid scale of the Tube Holder indicates [W3] position. (Refer to the following illustration)
Do not add WDB four or more times. The contamination might be caused the waste fluid touching the Cartridges, or the waste fluid might overflow from the Waste Tubes.



<5> <Elution> Pull out the Cartridge Holder and the Tube Holder and then insert the Cartridge Holder into the Elution Position (E) of the Tube Holder. Do not touch the Release Lever. Apply 200 µl of CDB to the Cartridges (CA) and then set the Cartridge Holder and the Tube Holder in QG-Mini80. After setting it, check the Wash Label cannot be seen by hiding under the Tube Holder. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no CDB remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CDB remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
The volume of CDB can be reduced to 50 µl, but in the case, elution efficiency might decrease by about 20%.



<6> Pull out the Cartridge Holder and the Tube Holder. Remove the Cartridge Holder from the Tube Holder and then dispose of the Cartridges (CA). When sliding the Release Lever to the right-hand end, all Cartridges will fall down. Remove the Collection Tubes and then insert the Collection Tubes into the Tube Rack (optional) and then put Caps (CAP).
When the Tube Rack is not used, remove the Collection Tubes after putting Caps on them.
When using commercially available 1.5 ml microtubes : Put caps on 1.5 ml microtubes and then remove them.
Dispose the Waste Tubes and waste fluid according to appropriate laws and rules.

The standard yield is 4 to 8 µg from 200 µl of whole blood samples.
Cover with the Caps (CAP) on the Collection Tubes (CT) tightly, 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.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene DNA whole blood kit S (DB-S).

(*) : For QG-810/QG-800
(**) : For QG-Mini80

(1) Low yield or no DNA obtained :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Inadequate dissolution of EDB	After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4°C. Do not use EDB preserved for a longer period than 2 months. 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.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Inappropriate volume of whole blood sample	If the volume of a whole blood sample is too much, reduce it to the prescribed volume (200 µl). Small amount of sample should be adjusted to 200 µl with PBS (sterilized) before loading.
Use of too much amount of leucocytes	The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6 cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.9)
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Insufficient volume of CDB (**)	Confirm the amount of CDB is 50 µl or more.
Rupture of filter	Be careful not to allow pipette tip to contact with a filter in the Cartridge (CA).
Perform exceed pressurization (**)	Stop applying air pressure as soon as lysate or WDB is discharged.
Leaving Cartridge (CA) after lysate or WDB are discharged (**)	During the procedure, work quickly without interruption.
Use of reagents other than CDB to elute genomic DNA	Use CDB to elute genomic DNA.
Use of too old WDB (*)	Check if WDB (>99% ethanol added) setted in QG-810/QG-800 does not pass over 1 day.
DNA degradation	Refer to (3) “DNA degradation”.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.

(2) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of a whole blood sample	Reduce it to the prescribed volume (200 µl).
Use of too much amount of leucocytes	The Cartridge (CA) might clog when the number of leucocytes exceeds 5×10^6 cells/200 µl. The yield of DNA might decrease when the number of leukocytes exceeds 2×10^6 cells/200 µl . In such case, we recommend that you dilute the sample with PBS (sterilized) to below 2×10^6 cells/200 µl, and then perform extraction.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).

(3) DNA degradation :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

(4) Purity of DNA is low :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4°C. Do not use EDB preserved for a longer period than 2 months. 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.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.9)

Cause	Action
Improper washing procedure (**)	Wash 3 times with 750 µl of WDB.
Use of reagents other than CDB to elute genomic DNA	Use CDB to elute genomic DNA.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Low purity of DNA	Refer to (4) “Purity of DNA is low”.
DNA degradation	Refer to (3) “DNA degradation”.

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at room temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 37°C. Cool down it to room temperature before use.

(7) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CDB or no operation of discharging (*)	Set the prescribed volume of CDB according to Table 3 (p.10). In addition, it is necessary to perform a discharging operation according to the User’s Guide of QG-810/QG-800.
Not addition of CDB (**)	After insert the Cartridge Holder to the Elution Positon (E), add 200 µl of CDB to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CDB (**)	When adding CDB, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(8) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder, and then set Cartridges (CA).

10. Ordering Information

Product	Cat #
QuickGene DNA tissue kit S	DT-S
For extraction of genomic DNA from tissues	
QuickGene DNA whole blood kit S	DB-S
For extraction of genomic DNA from whole blood	
QuickGene RNA tissue kit S II	RT-S2
For extraction of total RNA from tissues	
QuickGene RNA cultured cell kit S	RC-S
For extraction of total RNA from cultured cells	
QuickGene RNA cultured cell HC kit S	RC-S2
For extraction of total RNA from cultured cells	
QuickGene RNA blood cell kit S	RB-S
For extraction of total RNA from leukocytes	
QuickGene Plasmid kit S II	PL-S2
For extraction of plasmid DNA from *Escherichia coli*	

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Appendix 1 Setting of QG-810 Parameter

In the case of using QG-810, select “DNA WHOLE BLOOD” mode. The parameter of “DNA WHOLE BLOOD” is the following Table.

Display Sequence	LCD message	PARAMETER
1	BIND PEAK	120
2	WASH COUNT	3
3	WASH PEAK	110
4	WASH VOL1	750
5	WASH VOL2	750
6	WASH VOL3	750
7	WASH VOL4	750
8	WASH VOL5	750
9	WASH DIP TM	0
10	WAS2 WAIT T	0
11	WAS2 COUNT	0
12	WAS2 PEAK	110
13	WASH VOL1	750
14	WASH VOL2	750
15	WASH VOL3	750
16	WASH VOL4	750
17	WASH VOL5	750
18	ELUT VOL	200
19	ELUT PEAK	100
20	ELUT DIP TM	0

* When changing CDB volume to 50 µl, change “ELUT VOL” to “50”.
When changing the parameter, refer to QG-810 User’s Guide.

Appendix 2 Setting of QG-800 Parameter

In the case of using QG-800, select “DNA WHOLE BLOOD” mode. The parameter of “DNA WHOLE BLOOD” is the following Table.

Display Sequence	Operation menu	PARAMETER
1	SAMP PEAK	120
2	WASH COUNT	3
3	WASH PEAK	110
4	WASH VOL1	750
5	WASH VOL2	750
6	WASH VOL3	750
7	WASH VOL4	750
8	WASH VOL5	750
9	WAS2 COUNT	0
10	WAS2 PEAK	110
11	WAS2 VOL1	750
12	WAS2 VOL2	750
13	WAS2 VOL3	750
14	WAS2 VOL4	750
15	WAS2 VOL5	750
16	CLCT VOL	200
17	CLCT PEAK	120

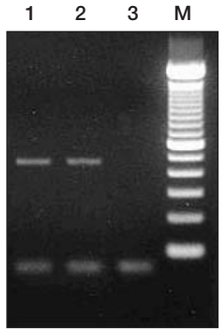
* When changing CDB volume to 50 µl, change “CLCT VOL” to “50”.
When changing the parameter, refer to QG-800 User’s Guide.

Appendix 3 Examples of the Data with QuickGene DNA whole blood kit S (DB-S)

● PCR

Figure 1 shows an example of PCR of genomic DNA extracted with this kit.
PCR was performed with 0.1 ng of genomic DNA extracted from 200 µl of a whole blood sample with this kit using G3PDH as a target.

Figure 1



No.	Sample
1	200 µl of a whole blood sample (Using QG-800)
2	200 µl of a whole blood sample (Using QG-Mini80)
3	Negative control

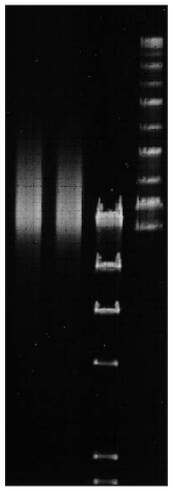
M : Marker (100 bp DNA Ladder : Invitrogen)
Erectrophoresis condition : 2% Agarose gel/1 × TAE

As a result of this PCR, the band of the amplification product from 0.1 ng of genomic DNA template was detected.

● Results of pulse field electrophoresis

Figure 2 shows the length of genomic DNA extracted with this kit.

Figure 2



No.	Sample
1	DNA extracted from 200 µl of a whole blood sample with this kit (Using QG-800) (<-140 kb)
2	DNA extracted from 200 µl of a whole blood sample with this kit (Using QG-Mini80) (<-140 kb)

M1 : λ-Hind Ⅲ digest
M2 : MidRange PFG Maker Ⅱ (NEB)
Erectrophoresis condition : 1% Agarose gel/0.5 × TBE

From the result, genomic DNA extracted with this kit has a length of less than 140 kb.

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DB-S_HB-E_V30