KURABO

HANDBOOK

QuickGene RNA blood cell kit S (RB-S)

For extraction of total RNA from leukocytes

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 total RNA 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 total RNA can be extracted and also purified from leukocytes. No hazardous organic solvents such as phenol and chloroform are used. RNA from 8 sets of cell lysate samples can be simultaneously extracted in following time.
QuickGene-810/QuickGene-800 (QG-810/QG-800) : about 20 min (without DNase treatment)
QuickGene-Mini80 (QG-Mini80) : about 15 min (without DNase treatment)
The purified, high quality total RNA is suitable for RT-PCR, Northern blot analysis 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)

☐ Lysis Buffer	LRB	75 ml
☐ Wash Buffer	WRB	280 ml
☐ Elution Buffer	CRB	100 ml
☐ Cartridges	CA2	96
☐ Collection Tubes	CT	96
☐ Caps	CAP	96
☐ Waste Tubes	WT	96

2-2 Storage Conditions

All reagents are stable at room temperature (15-28°C) untill expiring date idicated at outer box.

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

- 2-Mercaptoethanol (2-ME) (to be used as an additive to LRB)
- >99% Ethanol (for preparation of lysate and WRB working solution)

* Prepare if necessary

- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - DNase I, Amplification Grade (Life Technologies : Cat. No. 18068-015)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)
  - DNase I, Amplification Grade (Sigma-Aldrich : Cat. No. AMP-D1)

[2] Equipments

- QuickGene
- Centrifuge tubes * (Large/Small sets)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- 2.0 ml microtubes (RNase-free) (if needed)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Ball (zirconia, 5 mmϕ, if needed)
- Benchtop microcentrifuge

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

Recommendation products 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 WRB)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRB)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRB)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRB)	BD Falcon™ 50 ml conical tube

4. Safety Warnings

Warning For research use only.
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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/)

◆ LRB (Lysis Buffer)
- **Harmful if ingested.**
- Do not drink or ingest. Avoid contact with eyes.
- It should be handled at a well-ventilated place. Wear a laboratory coat, gloves and safety goggles during experiments.
- If contact with eyes, skin or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Use or storage of this buffer close to fire or at high temperature should be avoided.
- Keep the bottle tightly closed.

◆ WRB (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.

◆ CRB (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.

◆ Any solution and waste fluid containing LRB 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 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

- Do not use frozen blood samples.
- The yield varies depending upon sample conditions (health condition which blood derived from).
- In case clogging occurs, try by reducing the number of leukocytes.

◆ Use of Reagent

- If the precipitates are formed in LRB, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.
- Use or storage of LRB at high temperature should be avoided.
- Any solution and waste fluid containing LRB should not be mixed with bleach.

◆ Procedure of Extraction

- Use QuickGene RNA blood cell kit S (RB-S) at room temperature (15-28°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : 8-3 (p.13), Appendix 1 (p.29), Appendix 2 (p.31)
  QG-Mini80 : 8-4 (p.17)
- Refer to QuickGene User's Guide for the details.

<Prevention Against RNase Contamination>

- Wear disposable gloves when you are handling the RNA kit components or any other equipment, prevent RNase contamination.
- Use of sterile, disposable plasticware throughout the procedure is recommended. These plasticware are generally RNase-free and do not require pretreatment to inactivate RNases.
- Glassware or metal tools should be oven baked at 200°C for 16 hours or more.

6. Quality Control

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

7. Product Description

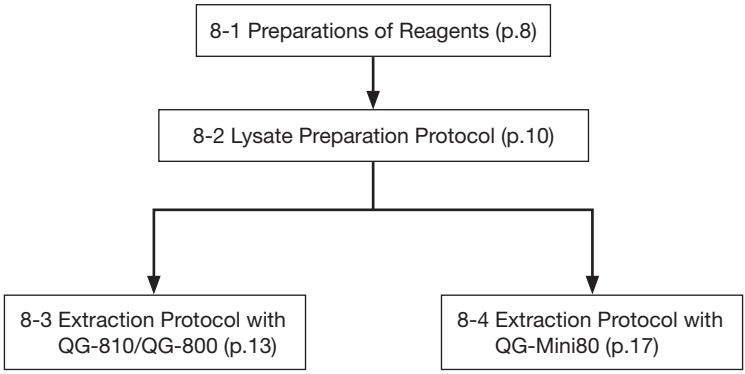
QuickGene RNA blood cell kit S (RB-S) corresponds to the extraction of total RNA from leucocytes (1.5×10^7 cells or less) after hemolysis. About 4,000-7,000 leucocytes are included in each µl of blood from healthy adults. Thus about 2 ml of the blood including 7,000 leukocytes/µl provides the maximum number of leucocytes (1.5×10^7 cells) suitable for this kit. Table 2 shows the example of yield and purity the total RNA extracted from different numbers of blood cells (in the case with DNase treatment).

Table 2 Yield and purity of total RNA

Number of leucocytes	Yield of total RNA recovered (µg)	A260/280
1×10^6	0.3	2.1
1×10^7	4.6	2.2
1.5×10^7	6.5	2.1

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

◆ LRB (75 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.

2-Mercaptoethanol (2-ME) must be added to LRB before each use. Add 10 µl 2-ME per 1 ml of LRB. Use 520 µl of LRB per 1 Cartridge (CA2). Dispense in a fume hood and wear appropriate protective clothing.

◆ WRB (280 ml)

WRB is supplied as a concentrate.

Add 120 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.

◆ CRB (100 ml)

Use CRB for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to the Details of Workflow (8-3 <3> p.15, 8-4 <3> p.20).

Use immediately after preparation.

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

Prepare the required volume of WRB and CRB into the tubes (see Table 1 p.4) : set them to Buffer Stand.

Table 3 Required volume of WRB and CRB

Number of Cartridges	WRB (QG-810/QG-800)	CRB (QG-810)	CRB (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 : WRB 8.0 ml, CRB 7.4 ml
QG-800 : WRB 8.0 ml, CRB 6.4 ml
Depending on the number of the Cartridges, add WRB and CRB.
Use WRB 2.25 ml and CRB 50 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WRB, 7.5 ml of CRB (QG-810) and 6.5 ml of CRB (QG-800) are required.
*Use appropriate tubes according to Table 1 (p.4).

8-2 Lysate Preparation Protocol

QuickGene RNA blood cell kit S (RB-S) corresponds to the extraction of total RNA from leucocytes (1.5×10^7 cells or less) after erythrocytes lysis.

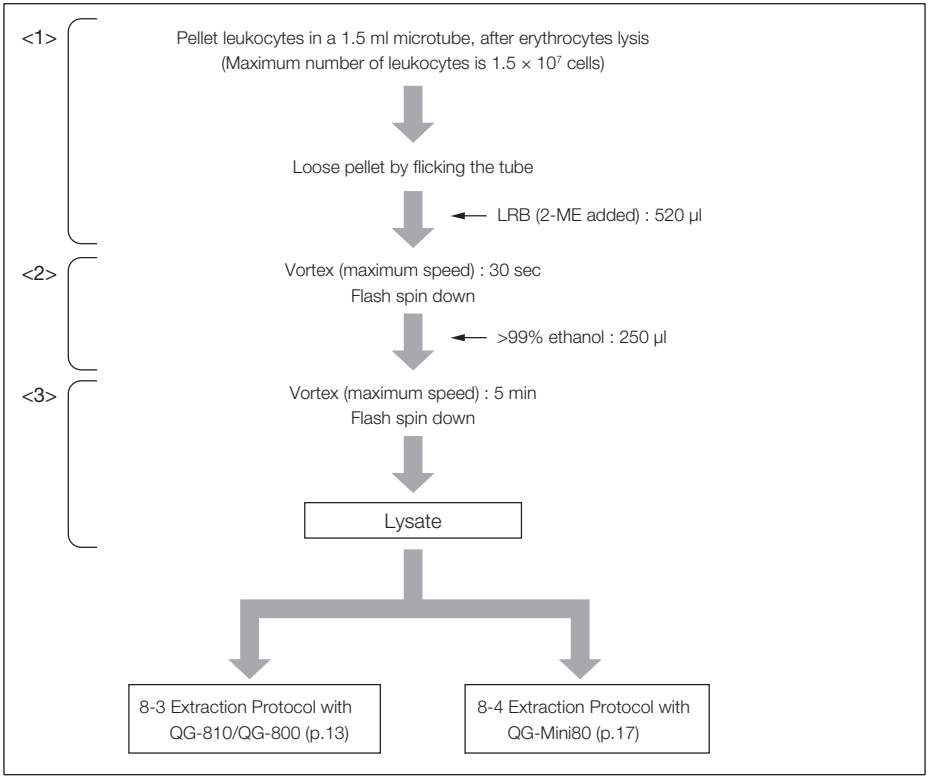
[Important Notes Before Starting]

- Cool down all reagents to room temperature before use.
- Count the number of leukocytes, confirm that it is under 1.5×10^7 cells before use.
  About 4,000-7,000 leucocytes are included in each µl of blood from healthy adults.
  For example, 2 ml of blood with containing 7,000 leukocytes/µl is proper for 1.5×10^7 cells per Cartridge (CA2).
- Do not use frozen blood samples.
- Follow the volume of samples and buffers described in the workflow (p.11).
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- Any solution and waste fluid containing LRB 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]

- WRB is supplied as a concentrate. Check that 120 ml of >99% ethanol is added to WRB before starting an experiment.

Lysate Preparation Workflow



Details of Lysate Preparation Workflow

<1> Use a pellet of leukocytes in a 1.5 ml microtube, after erythrocytes lysis.
In case of using excessive amounts leukocytes, extraction would end with the following results : clogging, low yield, and low purity. Please make sure the number of leukocytes is under 1.5 × 10^7 cells. When clogging occurs, reduce the number of cells, and then try again.
Add 2-ME to LRB before use (p.8).

<1a> Pelleted leukocytes (-1.5 × 10^7) in 1.5 ml microtube, after erythrocytes lysis :
Loosen the pelleted cells thoroughly by flicking the tubes. Add 520 µl of LRB (2-ME added). Mix LRB and cells thoroughly by pipetting.

<1b> Pelleted leukocytes (-1.5 × 10^7) in except 1.5 ml microtube, after erythrocytes lysis :
Loosen the pelleted cells thoroughly by flicking the tubes. Add 520 µl of LRB (2-ME added). Mix LRB and cells thoroughly by pipetting, and transfer into a 1.5ml microtube.

<2> Vortex at the maximum speed for 30 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

Make sure to vortex at the maximum speed for 30 sec.

<3> Add 250 µl of >99% ethanol. Vortex at the maximum speed for 5 min. Flash spin down for several seconds to remove drops from the inside of the lid.

As in step <2>, make sure to vortex at the maximum speed for 5 min.
Optional procedure : For the best result, it is recommended to add a ball (zirconia 5 mmϕ) during homogenization. When using a ball, use 2 ml microtube.

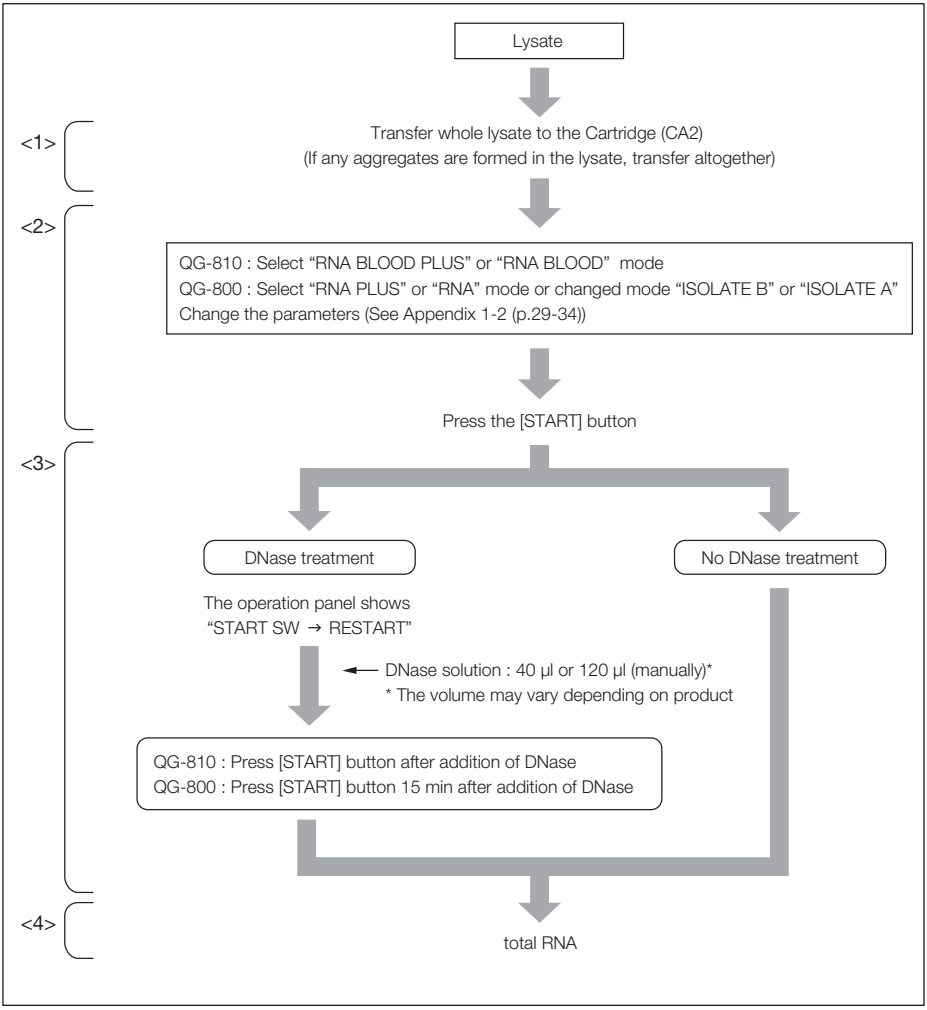
Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.13)
QG-Mini80 (p.17)

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 120 ml of >99% ethanol is added to WRB before starting an experiment.
- Select "RNA BLOOD PLUS" or "RNA BLOOD" mode of QG-810 for total RNA extraction (Appendix 1 p.29).
- Select "RNA PLUS" or "RNA" mode or changed mode "ISOLATE B" or "ISOLATE A" of QG-800 for total RNA extraction (Appendix 2 p.31).
- All reagents, Cartridges (CA2) 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 (CA2), tubes and each reagent.
- Open the front cover of QG-810/QG-800 and set the Collection Tubes (CT) and Waste Tubes (WT) in the Tube Holder (or Collection Tube Holder). Use the specified Cartridges (CA2).
- Set WRB (>99% ethanol added) and CRB to QG-810/QG-800 referring to p.9.
- Incorrect Cartridge (CA2) 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 (CA2) with the pipette tip.
- Any solution and waste fluid containing LRB 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.10) to the each Cartridge (CA2).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.

<2> <Extraction> Select the appropriate mode for this kit. In case of confirming the setting of parameter, refer to Appendix 1 (p.29), Appendix 2 (p.31). 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.5).

<3> <DNase treatment> If not using a DNase, proceed to <4>. Prepare the DNase solution according to the following tables.

<3-1> Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
DNase I, Amplification Grade	Life Technologies	18068-015		
RNase-Free DNase Set*1	QIAGEN	79254	2	3.4 Kunitz units/40 µl
DNase I, Amplification Grade	Sigma-Aldrich	AMP-D1	3	60 U/120 µl

*1 : Dissolve 1,500 Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

Preparation 1)

1 U/µl DNase I	20 µl
10 × Reaction Buffer	4 µl
Nuclease-Free water	16 µl

Preparation 2)

2.7 Kunitz units/µl DNase I*2	1.25 µl
Buffer RDD	35 µl
Nuclease-Free water	3.75 µl

*2 : The QIAGEN protocol may cause excess DNase activity. We recommend the method above.

Preparation 3)

1 U/µl DNase I	60 µl
10 × Reaction Buffer	12 µl
Nuclease-Free water	48 µl

<3-2> Method for DNase treatment on column

Confirm that the operation panel shows, “START SW → RESTART”, and then open the front cover. Add the DNase solution prepared at <3-1> directly onto a filter in each Cartridge (CA2). If using DNase from Promega, Invitrogen, or QIAGEN, add 40 µl of the prepared DNase solution per Cartridge evenly over the filter. If using DNase from Sigma, add 120 µl of the prepared DNase solution per Cartridge.

* Avoid touching the filter in the Cartridge with the pipette tip during the addition of DNase solution.
In the case of using QG-810, proceed to <3-2a>, whereas in the case of using QG-800, proceed to <3-2b>.

<3-2a> For QG-810

It is easy to see the pipette tip from back of the Holder Carriage. Reset the Holder Carriage to the original place after DNase addition.
Close the front cover, and press [START] button. Extraction operation starts automatically after 15 min (the operation panel shows “PROCESSING”). Default waiting (holding) time of DNase treatment is 15 min. You can change the setting of time as the parameter of a program. (Parameter of “WAS2 WAIT T” see Appendix1, p.29)

<3-2b> For QG-800

Close the front cover, and incubate at room temperature on the Cartridge for 15 min. Press the [START] button to restart the extraction process (the operation panel shows “EXECUTING”).

<4> <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 (CA2) will be 50 µl.

If you do not use the eluted total RNA for any experiments immediately, after covering the Caps (CAP) on the Collection Tubes (CT) tightly, store at –20°C or –80°C.

<5> Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.
Remove the Cartridge Holder and dispose the Cartridges (CA2).
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 120 ml of >99% ethanol is added to WRB before starting an experiment.
- Set Waste Tubes (WT) into the Tube Holder.
- Set Tube Adapters to the Tube Holder, and set the 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 (CA2) to the Cartridge Holder. Confirm the Release Lever is positioned at the left-hand end of the Cartridge Holder.

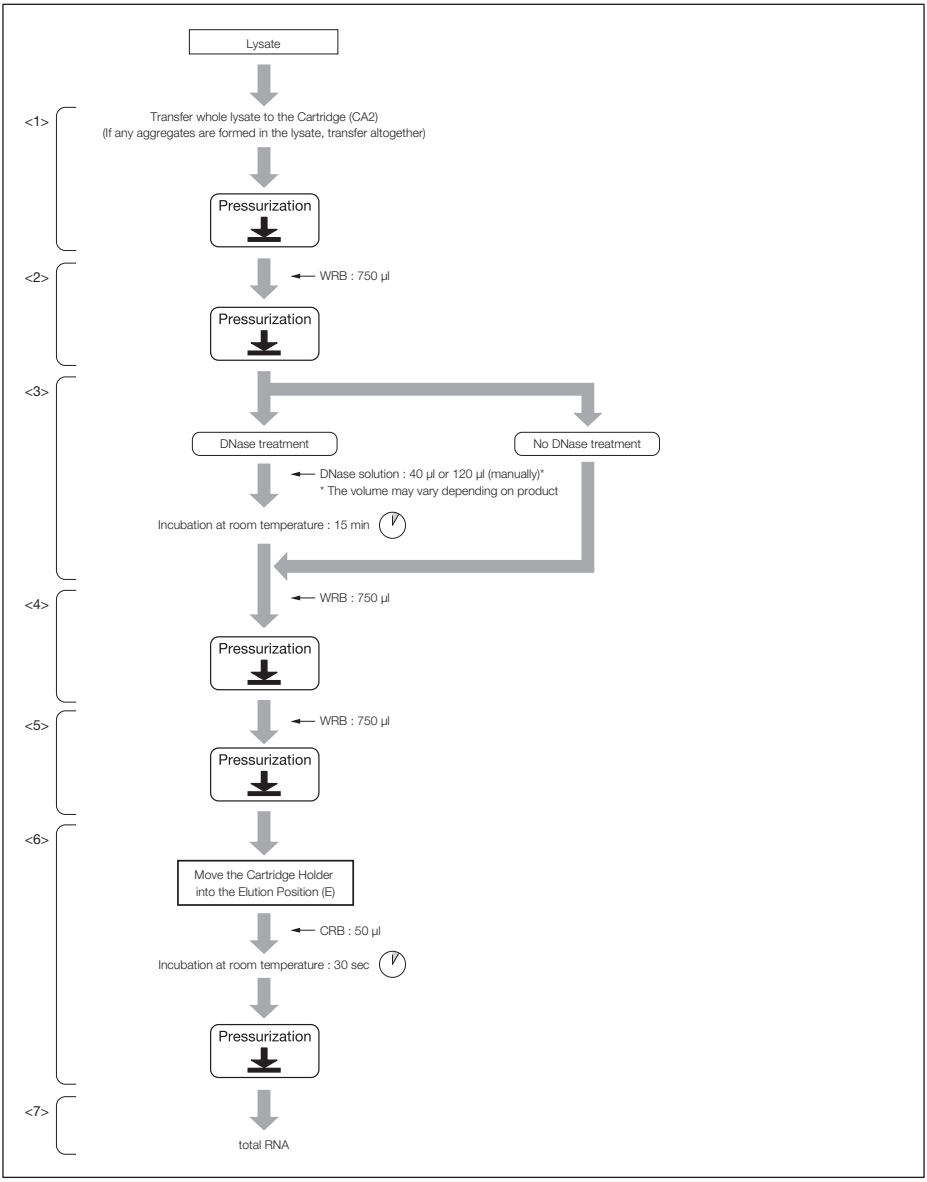
- When setting the Cartridge Holder and the Tube Holder to QG-Mini80, insert to the end.
- When pressuring lysates and WRB (>99% ethanol added), confirm that the Wash Label on the Tray can be entirely seen.
- When pressuring CRB, confirm that the Wash Label on the Tray can not be seen, being hidden below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

- Avoid touching the filter in the Cartridge (CA2) with the pipette tip.
- Any solution and waste fluid containing LRB should not mixed with bleach.
- When using potentially infectious samples for experiments, dispose 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 (CA2) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.



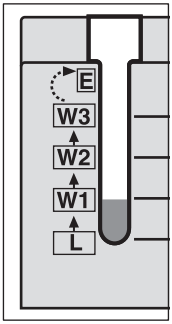
Details of QG-Mini80 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate prepared at 8-2 (p.10) to each Cartridge (CA2). 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 Cartridges.
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 WRB to the Cartridges (CA2). 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 WRB remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WRB 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 first wash, the waste fluid scale of the Tube Holder indicates [W1] position. (Refer to the following illustration)



<3> <DNase treatment> If not using a DNase, proceed to <4>. Prepare DNase solution according to the following tables.

<3-1> Prepare the recommended DNase

Product name	Manufacture	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
DNase I, Amplification Grade	Life Technologies	18068-015		
RNase-Free DNase Set*1	QIAGEN	79254	2	3.4 Kunitz units/40 µl
DNase I, Amplification Grade	Sigma-Aldrich	AMP-D1	3	60 U/120 µl

*1 : Dissolve 1,500 Kunitz units of DNase with 550 µl of RNase-Free water (attached) before preparing the DNase reaction solution.

Preparation 1)

1 U/µl DNase I	20 µl
10 × Reaction Buffer	4 µl
Nuclease-Free water	16 µl

Preparation 2)

2.7 Kunitz units/µl DNase I*2	1.25 µl
Buffer RDD	35 µl
Nuclease-Free water	3.75 µl

*2 : The QIAGEN protocol may cause excess DNase activity. We recommend the method above.

Preparation 3)

1 U/µl DNase I	60 µl
10 × Reaction Buffer	12 µl
Nuclease-Free water	48 µl

<3-2> Method for DNase treatment on column

Pull out the Cartridge Holder and the Tube Holder. In case of DNase preparation of Promega, Invitrogen, QIAGEN, add 40 µl per Cartridge (CA2) evenly over the filter. In case of DNase preparation of Sigma, add 120 µl per Cartridge evenly over the filter. After addition, set the Cartridge Holder and the Tube Holder into QG-Mini80 and incubate the Cartridge at room temperature for 15 min.

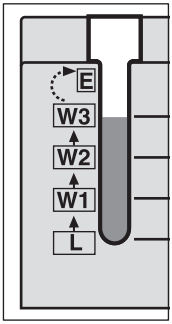
Avoid touching the filter in the Cartridge with the pipette tip during addition of DNase solution.
Pressurization should not be performed during incubation.
It is necessary to start pressurization after addition of WRB for the second wash (<4>).
It is necessary to start DNase treatment after the first wash.

<4> <Second wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WRB to the Cartridges (CA2). 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 WRB remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WRB 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.

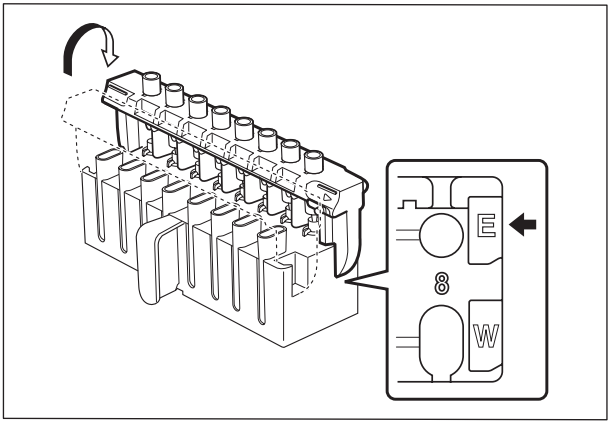
<5> <Third wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WRB to the Cartridges (CA2). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen.
Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WRB remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WRB 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 WRB 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.



<6> <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 50 µl of CRB to the Cartridges (CA2) 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.
Incubate the Cartridge for 30 sec after applying CRB. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no CRB remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CRB 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.



<7> Pull out the Cartridge Holder and the Tube Holder. Remove the Cartridge Holder from the Tube Holder and then dispose the Cartridges (CA2). 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.

If you do not use the eluted total RNA for any experiments immediately, after covering the Caps (CAP) on the Collection Tubes (CT) or caps of 1.5 ml microtubes tightly, store at –20°C or –80°C.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA blood cell kit S (RB-S).

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

(1) Low yield or no RNA obtained :

Cause	Action
No addition of 2-ME to LRB	Dispense a required volume of LRB before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRB.
Insufficient lysis of leukocyte	Check that there is no precipitate in LRB. In case a precipitate is formed, dissolve the precipitate by incubation at 37°C. Use it after cooling back to room temprature.
Insufficient vortexing after addition of LRB (2-ME added)	Vortex thoroughly (See section 8-2 p.10).
No addition of the prescribed volume of ethanol to WRB	Before using WRB for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See 8-1 p.8)
Incomplete addition of whole lysate to the Cartridge (CA2)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Insufficient volume of CRB	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of “ELUT VOL” (QG-810) or “CLCT VOL” (QG-800) is correct (it should be “50”). In addition, in case air bubbles still remain in the line of QG-810/ QG-800, discharge them. For setting of parameter, refer to User’s Guide of QG-810/ QG-800. QG-Mini80 : Confirm the amount of CRB is 50 µl.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.
Leaving Cartridges (CA2) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.
Use of reagents other than CRB to elute RNA	Use CRB to elute RNA.
Use of too old WRB (*)	Check if WRB (>99% ethanol added) setted in QG-810/QG-800 does not pass over 1 day.
No incubation performed at the time of elution	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of “ELUT DIP TM” (QG-810) or “CLCT DIP TM” (QG-800) is correct (it should be “30”). For setting of parameter, refer to User’s Guide of QG-810/QG-800. QG-Mini80 : Incubate for 30 sec after addition of CRB onto the filter.
No addition of the prescribed volume of DNase Reaction Buffer (When using a DNase)	When using a DNase, check that the prescribed volume of DNase Reaction Buffer has been added.
Perform pressurization without adding WRB to the Cartridge (CA2) after the 15 min incubation following the addition of a DNase solution (**)	Add a DNase solution, incubate for 15 min, then add WRB to the Cartridge before pressurization.
Rupturing of filter when adding a DNase (when using a DNase)	Add a DNase solution not so as to allow the end of tip to contact with filter. In case of QG-810, take Holder Carriage off, add DNase solution with confirming the end of tip from backside.
RNA degradation	Refer to (3) “RNA degradation”.

Cause	Action
Temperature of operation is high	Take all of operation at room temperature (15-28°C).
Clogged filter (Operation panel of QG-810 : –, QG-800 : ×)	Take a filter out of the clogged Cartridge (CA2) and try the recovery of RNA according to "Further Note" p.27.

(2) Clogging of Cartridge (CA2) occurs :

Cause	Action
Insufficient vortexing after addition of LRB or ethanol	After LRB or ethanol addition, perform vortexing completely for 30 sec or 5 min, respectively, according to section 8-2 (p.11). Pipette several times when transferring lysate to the Cartridge (CA2). Optionally put a ball (zirconia 5 mmϕ) in a 2 ml tube before vortexing for 5 min after ethanol addition.
Use of too much amount of leukocytes	Reduce the amount of leukocytes.
Inadequate lysis of leukocytes	After LRB or ethanol addition, perform vortexing completely for 30 sec or 5 min, respectively.
Insufficient pressurization (**)	Pressurize once more.
Leaving Cartridges (CA2) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.
QG-810/QG-800 : Operation panel of " – (QG-810)" or "× (QG-800)" is displayed, and lysate or WRB is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRB completely despite repeated pressurization (**)	Do DNase treatment, and try the recovery of RNA according to Further Note (p.27).
No addition of the prescribed volume of ethanol to WRB	Before using WRB for the first time, ensure that the prescribed volume of >99% ethanol has been added (See 8-1 p.8).

(3) RNA degradation :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use only fresh blood. Frozen whole blood cannot be used. After erythrocytes lysis, all steps of this protocol should be performed as quickly as possible.
No addition of 2-ME to LRB	Dispense a required volume of LRB before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRB.
RNase contamination	Although all buffers, Cartridges (CA2), Collection Tubes (CT) and Caps (CAP) are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of the recommended RNase-free DNase. For the details, inquire to each manufacture.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(4) Subsequent experiments such as RT-PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of RNA is used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination with genomic DNA	Perform the DNase treatment by selecting the mode “RNA BLOOD PLUS” (QG-810) or either “RNA PLUS” or “ISOLATE B” (QG-800). Refer to the following (5) when the degradation of DNA is insufficient.
RNA degradation	Refer to (3) “RNA degradation”.
Severe contamination with foreign matter (**)	After the first addition of WRB, incubate at room temperature for 2 min. Incubation during the second and third wash should not be required.
No use of prescribed washing condition	QG-810/QG-800 : Confirm that the parameter “WASH VOL 1-5” and “WAS2 VOL 1-5” is “750” ; refer to Appendix 1 and 2 (p.29-34). QG-Mini80 : Wash the filter three times with 750 µl of WRB.

(5) Imperfect degradation of DNA (when using a DNase) :

Cause	Action
Use of DNase other than the recommended DNase	Use a recommended DNase (See section 8-3<3> p.15, 8-4<3> p.20).
Filter was not completely soaked in DNase solution	Make sure that DNase is evenly covered over the filter in the Cartridge (CA2) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	QG-810 : Confirm that the parameter “WAS2 WAIT T” is “15”. QG-800 or QG-Mini80 : Incubate at room temperature (15-28°C) for 15 min.
Required volume of DNase is not added	When preparing a DNase solution, check that the prescribed amount of DNase has been added.

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 37°C. Use it after cooling back to room temperature.

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

Cause	Action
Insufficient set of CRB or no operation of discharging (*)	Set the prescribed volume of CRB according to Table 3 (p.9). In addition, it is necessary to perform a discharging operation according to the User’s Guide of QG-810/QG-800.
No addition of CRB (**)	After insert the Cartridge Holder to the Elution Position (E), add 50 µl of CRB to Cartridge (CA2).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CRB (**)	When adding CRB, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(8) Cartridge (CA2) 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 (CA2).

Further Note : Method for Recovering RNA from Clogged Cartridge (CA2)

In case of QG-810 or QG-800 :
Leave the fluid remaining in the Cartridge (CA2) as it stands and then add DNase solution according to the method described in section 8-3 <3> (p.15). The DNase should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip.
Close the front cover and then leave the instrument standing at room temperature for 15 min. Then, check the mode, and press the [START] button to begin the first step.

In case of QG-Mini80 :

<1> If clogging occurs at the lysate pressurization step

<1-1> Clogging during lysate pressurization
Leave the lysate remaining in the Cartridge (CA2) as it stands, add DNase solution is according to the method described in section 8-4 <3> (p.20). The DNase solution should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip. The DNase treatment is 15 min at room temperature.
Rotate the Rotary Switch of QG-Mini80 toward the front side to begin pressurization. After checking that no lysate remains in the Cartridge, perform the operation from 8-4 <2> (p.19).

If complete removal of DNA is required, include a DNase treatment as per normal after the first wash step (see section 8-4 <3> p.20).

<1-2> After <1-1>, clogging during the first wash step
Leave the WRB remaining in the Cartridge (CA2) as it stands, add DNase solution is according to the method described in section 8-4 <3> (p.20). The DNase solution should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip. The DNase treatment is 15 min at room temperature.
Rotate the Rotary Switch of QG-Mini80 toward the front side to begin pressurization. After checking that no WRB remains in the Cartridge, perform the operation from 8-4 <4> (p.21).

<2> If clogging occurs at the washing step :
Leave the WRB remaining in the Cartridge (CA2) as it stands, add DNase solution is according to the method described in section 8-4 <3> (p.20). The DNase solution should be added as close as possible to the filter. Avoid touching the filter in the Cartridge with the pipette tip. The DNase treatment is 15 min at room temperature.
Rotate the Rotary Switch of QG-Mini80 toward the front side to begin pressurization.
After checking that no WRB remains in the Cartridge, perform the operation from 8-4 <4> (p.21).

If complete removal of DNA is required, add DNase solution again after passage of the WRB as described in section 8-4 <3> p.20. The DNase treatment is 15 min at room temperature. Perform the operation from the second wash (see section 8-4 <4> p.21).

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*	

Appendix 1 Setting of QG-810 Parameter

In the case of using the QG-810, select the two unused modes, and change the name of extraction mode in “RENAME” mode.
Change the parameter to “RNA BLOOD PLUS” (in the case of DNase treatment), or “RNA BLOOD” (in the case of no DNase treatment).
However, the parameter is not changed only by changing the name of extraction mode.
The operating parameters need to be changed as shown in the table below.
When changing “RNA BLOOD PLUS”, make sure to change the parameter of “WAS2 WAIT T” as well.
When changing the parameter, refer to QG-810 User’s Guide.

* Gray-colored lines indicate parameters which do not need to be changed from the default values.

Display Sequence	LCD message	RNA BLOOD PLUS (With DNase treatment)		RNA BLOOD (Without DNase treatment)		Mode Default						
		PARAMETER	Check by user	PARAMETER	Check by user	DNA WHOLE BLOOD	RNA CELL	RNA CELL PLUS	DNA TISSUE	RNA TISSUE	RNA TISSUE PLUS	PLASMID
1	BIND PEAK	120		120		120	120	120	120	120	120	120
2	WASH COUNT	1		3		3	3	1	3	3	1	2
3	WASH PEAK	110		110		110	110	110	110	110	110	110
4	WASH VOL1	750		750		750	500	500	750	750	750	750
5	WASH VOL2	750		750		750	500	500	750	750	750	750
6	WASH VOL3	750		750		750	500	500	750	750	750	750
7	WASH VOL4	750		750		750	500	500	750	750	750	750
8	WASH VOL5	750		750		750	500	500	750	750	750	750
9	WASH DIP TM	150		150		0	150	150	0	150	150	0
10	WAS2 WAIT T	15		0		0	0	5	0	0	5	0
11	WAS2 COUNT	2		0		0	0	2	0	0	2	0
12	WAS2 PEAK	110		110		110	110	110	110	110	110	110
13	WASH VOL1	750		750		750	500	500	750	750	750	750
14	WASH VOL2	750		750		750	500	500	750	750	750	750
15	WASH VOL3	750		750		750	500	500	750	750	750	750
16	WASH VOL4	750		750		750	500	500	750	750	750	750
17	WASH VOL5	750		750		750	500	500	750	750	750	750
18	ELUT VOL	50		50		200	100	100	200	100	100	50
19	ELUT PEAK	100		100		100	100	100	100	100	100	100
20	ELUT DIP TM	30		30		0	30	30	90	30	30	0

<How to change parameters>

1. Select the extraction mode.
   Press the [MODE] button several times until “RNA BLOOD PLUS” or “RNA BLOOD” mode appears.

2. Press the [▲] [▼] button simultaneously.
   <Example of messages displayed in operation panel>

   SETUP START
   ↓
   RNA BLOOD
   The present mode is displayed.
   ↓
   BIND PEAK : 120*

   - The first item in operation menu and present set vaule are displayed.
   - Right number is current set value.
   - * is the present set value.

3. Press the [MODE] button several times until the target parameter appears.
   In the case of changing CRB volume “ELUT VOL” is displayed.
   To return to the previous parameter, press the [DISCHARGE] button.

4. Change the parameter settings using the [▲] [▼] buttons.
   -[▲] button : raise the setting value.
   -[▼] button : lower the setting value.
   <Example of operating for changing parameter>
   “ELUT VOL” change to “50” :
   “ELUT VOL” appears by [MODE] button → change to “50”

5. Press the [START] button then save the changed parameters.
   <Example of operation panel displays>

   SETUP WRITING
   Save setting content.
   ↓
   SETUP COMPLETED
   ↓
   RNA BLOOD
   Returns to ready status.

Appendix 2 Setting of QG-800 Parameter

A. In the case of using "RNA PLUS" or "RNA" mode

When using a QG-800, select either "RNA PLUS" or "RNA" modes.

To use "RNA PLUS" or "RNA" modes, the operating parameters need to be changed as shown in the table below.

When changing the parameter, refer to QG-800 User's Guide.

* Gray-colored lines indicate parameters which do not need to be changed from the default values.

Display Sequence	Operation menu	With DNase treatment			Without DNase treatment		
		PARAMETER	Check by user	"RNA PLUS" mode default	PARAMETER	Check by user	"RNA" mode default
1	SAMP SPEED	10		10	10		10
2	SAMP PEAK	120		120	120		120
3	SAMP UP TIME	10		10	10		10
4	SAMP RETRY	160		160	160		160
5	SAMP LOWER	75		75	75		75
6	SAMP DOWN TM	25		25	25		25
7	SAMP R DN T	50		50	50		50
8	SAMP FALL	50		50	50		50
9	WASH COUNT	1		1	3		3
10	WASH SPEED	3		3	3		3
11	WASH PEAK	110		110	110		110
12	WASH UP TIME	10		10	10		10
13	WASH RETRY	140		140	140		140
14	WASH LOWER	70		70	70		70
15	WASH DOWN TM	15		15	15		15
16	WASH R DN T	50		50	50		50
17	WASH FALL	50		50	50		50
18	WASH VOL1	750		500	750		500
19	WASH VOL2	750		500	750		500
20	WASH VOL3	750		500	750		500
21	WASH VOL4	750		500	750		500
22	WASH VOL5	750		500	750		500
23	WASH DIP TM	150		150	150		150
24	WAS2 COUNT	2		2	0		0
25	WAS2 SPEED	3		3	3		3
26	WAS2 PEAK	110		110	110		110
27	WAS2 UP TIME	10		10	10		10
28	WAS2 RETRY	140		140	140		140
29	WAS2 LOWER	70		70	70		70
30	WAS2 DOWN TM	15		15	15		15
31	WAS2 R DN T	50		50	50		50
32	WAS2 FALL	50		50	50		50
33	WAS2 VOL1	750		500	750		500
34	WAS2 VOL2	750		500	750		500
35	WAS2 VOL3	750		500	750		500
36	WAS2 VOL4	750		500	750		500
37	WAS2 VOL5	750		500	750		500
38	CLCT VOL	50		100	50		100
39	CLCT COUNT	1		1	1		1
40	CLCT SPEED	5		5	5		5
41	CLCT PEAK	120		120	120		120
42	CLCT UP TIME	20		20	20		20
43	CLCT RETRY	160		160	160		160
44	CLCT LOWER	65		65	65		65
45	CLCT DOWN TM	15		15	15		15
46	CLCT R DN T	50		50	50		50
47	CLCT FALL	50		50	50		50
48	CLCT DIP TM	30		30	30		30

B. In the case of using “ISOLATE B” or “ISOLATE A”

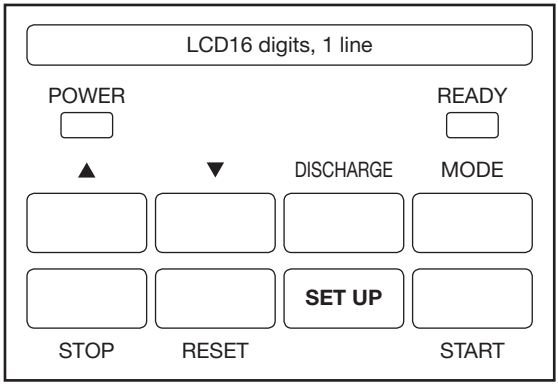
When using a QG-800, the parameter can be changed to “ISOLATE B” as the mode of DNase treatment, and “ISOLATE A” as the mode of no DNase treatment.

* Gray-colored lines indicate parameters which do not need to be changed from the default values.

Display Sequence	Operation menu	With DNase treatment			Without DNase treatment		
		PARAMETER	Check by user	“ISOLATE B” mode default	PARAMETER	Check by user	“ISOLATE A” mode default
1	SAMP SPEED	10		10	10		10
2	SAMP PEAK	120		120	120		120
3	SAMP UP TIME	10		10	10		10
4	SAMP RETRY	160		160	160		160
5	SAMP LOWER	75		75	75		75
6	SAMP DOWN TM	25		25	25		25
7	SAMP R DN T	50		50	50		50
8	SAMP FALL	50		50	50		50
9	WASH COUNT	1		3	3		3
10	WASH SPEED	3		3	3		3
11	WASH PEAK	110		110	110		110
12	WASH UP TIME	10		10	10		10
13	WASH RETRY	140		140	140		140
14	WASH LOWER	70		70	70		70
15	WASH DOWN TM	15		15	15		15
16	WASH R DN T	50		50	50		50
17	WASH FALL	50		50	50		50
18	WASH VOL1	750		750	750		750
19	WASH VOL2	750		750	750		750
20	WASH VOL3	750		750	750		750
21	WASH VOL4	750		750	750		750
22	WASH VOL5	750		750	750		750
23	WASH DIP TM	150		0	150		0
24	WAS2 COUNT	2		0	0		0
25	WAS2 SPEED	3		3	3		3
26	WAS2 PEAK	110		110	110		110
27	WAS2 UP TIME	10		10	10		10
28	WAS2 RETRY	140		140	140		140
29	WAS2 LOWER	70		70	70		70
30	WAS2 DOWN TM	15		15	15		15
31	WAS2 R DN T	50		50	50		50
32	WAS2 FALL	50		50	50		50
33	WAS2 VOL1	750		750	750		750
34	WAS2 VOL2	750		750	750		750
35	WAS2 VOL3	750		750	750		750
36	WAS2 VOL4	750		750	750		750
37	WAS2 VOL5	750		750	750		750
38	CLCT VOL	50		200	50		200
39	CLCT COUNT	1		1	1		1
40	CLCT SPEED	5		5	5		5
41	CLCT PEAK	120		120	120		120
42	CLCT UP TIME	20		20	20		20
43	CLCT RETRY	160		140	160		140
44	CLCT LOWER	65		65	65		65
45	CLCT DOWN TM	15		15	15		15
46	CLCT R DN T	50		50	50		50
47	CLCT FALL	50		50	50		50
48	CLCT DIP TM	30		0	30		0

<Changing the operating parameters>

1. Switching to “MAINTE MODE”
   1) Turn the power on while pressing the [START] and [▼] buttons.
   2) Release the [START] and [▼] buttons after the operation panel displays “TP MODE.”
   3) When the operation panel has displayed “TPOO : SENSOR TEST”, push the [▲] or [▼] button to change the initial “O” into “F.” Then, press the [MODE] button, to change the next “O” into “B.” Finally, “TPFB” is displayed.
   4) During the steps above, the display indicates “TPFB : SETUP MENU.”
   5) Press the [START] button to display “MENU : USER MODE.” Then, press the [▲] button to display “MENU : MAINTE MODE.”
   6) While “MENU : MAINTE MODE” is displayed, press the [RESET] button and [SET UP] button (the unnamed button located between the [START] and [RESET] buttons) simultaneously. The “READY” lamp soon begins to go on and off. When the lamp has gone on and off for 3 cycles, turn the power off and then turn the power on again.
   7) With the power is on, press [MODE] button to display the mode which requires changes to the operating parameters (e.g., “ISOLATE A”).
   8) If the [SET UP] button (the unnamed button between [RESET] and [START] buttons) is pressed, “SETUP START” will be displayed. About 1 sec later, the current mode (e.g., “ISOLATE A”) is displayed. About another sec later, the first of the operating parameters used and its current value are displayed. “✻” at the end indicates the current value.



2. Press [MODE] button for necessary times to display the desired parameter.
   [DISCHARGE] button is pressed to return to the previous parameter.
   After the last parameter, the display returns to the first parameter.
   Change the parameter into the level listed in the “Parameter” shown in the table (p.30, 31).

3. The parameter level is changed by pushing [▲] or [▼] button.
   Pressing [▲] button elevates the level, while pressing [▼] button reduces the level of the parameter.
   If either [▲] or [▼] button is kept compressed, the level will change serially.
   An attempt to elevate the level from the maximum level will cause the level to be set at the minimum level. An attempt to reduce the level from the minimum level will cause the level to be set at the maximum level.

4. If some other parameters need to be changed after one parameter has been changed, repeat steps 2 and 3.

5. Press [SET UP] button (the unnamed button between [RESET] and [START] buttons).
   The operation panel displays “SETUP WRITING” for about 1 sec and the level set is saved.
   The operation panel then displays “SETUP FINISH” for about 1 sec, and returns to the standby mode.
   ✻ If the level entered during setup needs to be cancelled, press [STOP] button. Cancellation of entered information is also possible by turning power off.

6. Return to “USER MODE”
   1) Turn power off.
   2) Take steps 1-1) through 1-4).
   3) Press [START] button to display “MENU : MAINTE MODE.” Then, press [▼] button to display “MENU : USER MODE.”
   4) While “MENU : USER MODE” is displayed, press [RESET] button and [SET UP] button (the unnamed button between [RESET] and [START] buttons) simultaneously. Soon the “READY” lamp begins to go on and off for 3 cycles.
   5) Turn power off, and then turn power on again. If power is on, the designated mode (e.g., “ISOLATE A”) is displayed, and standby mode is resumed.
      ✻ This step is needed to return to standby mode.

Appendix 3 Erythrocyte Lysis

We introduce a hemolysis method carrying out as an example in our company.

Hemolytic agent (HB)

NH_4Cl	150 mM
$NaHCO_3$	10 mM
EDTA (pH8.0)	0.1 mM

1. Mix 1 volume of whole human blood with 5 volumes of HB in an appropriately sized tube (not provided).

   For example, add 5 ml of HB to 1 ml of whole blood and mix well.
   Notice: Use an appropriate amount of whole blood. Up to 1.5×10^7 leukocytes of healthy blood (typically 4,000-7,000 leukocytes per µl) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used.

2. Incubate for 10-15 min on ice. Mix by inverting tubes or vortexing briefly 2 times during incubation.

   The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 min.

3. Centrifuge at 2,000 × g for 2 min at 4°C, and completely remove and discard supernatant.

   Leukocytes will form a pellet after centrifugation. Remove supernatant carefully, do not disturb pellet.

4. Add HB to the cell pellet (use 2 volumes of HB per volume of whole blood used in step 1. Resuspend cells by vortexing well.

   For example, add 2 ml of HB per 1 ml of whole blood used in step 1.
   Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in this wash step. If erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5-10 min on ice after addition of HB at this step.

5. Centrifuge at 2,000 × g for 2 min at 4°C, and completely remove and discard supernatant.

According to protocol, advance to the extraction step.

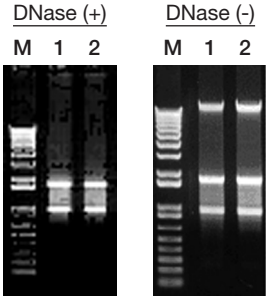
✻ After erythrocyte lysis, all of the extraction step should be performed as quickly as possible.

Appendix 4 Examples of the Data with QuickGene RNA blood cell kit S (RB-S)

● **Electrophoresis for total RNA (non denaturing gel electrophoresis)**

Figure 1 shows the result of electrophoresis of total RNA extracted with this kit.

Figure 1



No.	Sample
1	WBC (1×10^7)
2	WBC (1×10^7)

M : Marker (1 Kb Plus DNA Ladder : Life Technologies)
Electrophoresis condition : 1% Agarose/1 × TAE

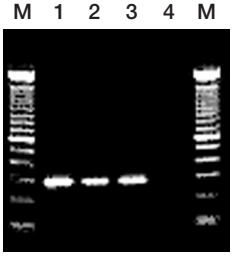
● **RT-PCR**

Figure 2 shows that the result of RT-PCR amplification, which performed using diluted total RNA extracted with this kit. RT-PCR was performed with GAPDH mRNA at following condition.

<RT condition>
Template : total RNA 5 µl
Kit : LightCycler 1st Strand cDNA Synthesis Kit for RT-PCR
LightCycler FastStart DNA Master SYBR Green I
LightCycler Human GAPDH Primer Set

<PCR condition>
Template : cDNA (1/125 of total RNA)
Primer : GAPDH primer

Figure 2



No.	Sample
1	Positive Control
2	WBC (5×10^5)
3	WBC (5×10^5)
4	Negative Control

M : Marker (100 bp DNA Ladder : Life Technologies)
Electrophoresis condition : 2% Agarose/1 × TAE

RT-PCR amplification was performed successfully using total RNA from 5×10^5 leucocytes.

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KURABO

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RB-S_HB-E_V30