KURABO

HANDBOOK

QuickGene RNA blood cell kit S (RB-S)

For extraction of total RNA from leukocytes

Ver.4.2

Contents

Warning

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

1. Introduction

This is a reagent kit for the extraction process of QuickGene-810 (Hereinafter QG-810) or QuickGene-Mini480 (Hereinafter QG-Mini480). When using this kit with QG-810 or QG-Mini480, high quality and high yield total RNA can be extracted and also purified from maximum 1.5 x 10^7 leukocytes.

The key features of this kit are described below;

- RNA from leukocyte can be simultaneously extracted in following time.
  QG-810: about 20 min for 8 sets. (Without DNase treatment)
  QG-Mini480: about 70 min for 48 sets.
- The purified, high quality total RNA is suitable for RT-PCR and northern blotting.

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)

Confirm that following contents is packed.

There are 96 reactions of total RNA isolation reagent and consumables.

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

2 - 2. Storage Conditions

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

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

- 0.5mol/L TCEP Solution (Neutral pH) (Used with LRB)
  [Recommended product] 0.5mol/L TCEP Solution, Neutral pH
  (FUJIFILM Wako Pure Chemical Corporation; Cat. No.207-20151)
- Ethanol (>99%) (For lysate preparation and WRB preparation)

*Prepare if necessary

- DNase
  [Recommended products]
  ・RQ1 RNase-Free DNase （Promega：Cat. No. M6101）
  ・DNase I, Amplification Grade （Thermo Fisher Scientific：Cat. No. 18068-015）
  ・RNase-Free DNase Set （QIAGEN：Cat. No. 79254）
  ・DNase I, Amplification Grade （Sigma-Aldrich：Cat. No. AMP-D1）

[2] Equipment

- 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
- Tube mixer (maximum speed at 2,500 rpm or more)
- Ball (zirconia, 5mmϕ, if needed)
- Benchtop microcentrifuge
  *Centrifuge tubes are used with QG-810 as containers for WRB (>99% ethanol added) and CRB. They are unnecessary when QG-Mini480 is used.

Recommendation products of centrifuge tubes are following Table 1.
Use centrifuge tubes according to the number of Cartridges to use.
Table1 Recommended centrifuge tubes (In case of QG-810)

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

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

◆ 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.

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

◆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 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

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

◆ Use of Reagent

- If the precipitates are formed in LRB, dissolve them fully by incubation at 37℃. 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 kit S (RB-S) at room temperature (15-28℃). 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: 8-3（p.13）, Appendix 1（p.29）
  QG-Mini480: 8-4（p.18）

  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 plastic ware 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 kit S (RB-S) is evaluated routinely on a lot-to-lot uniformity.
- 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

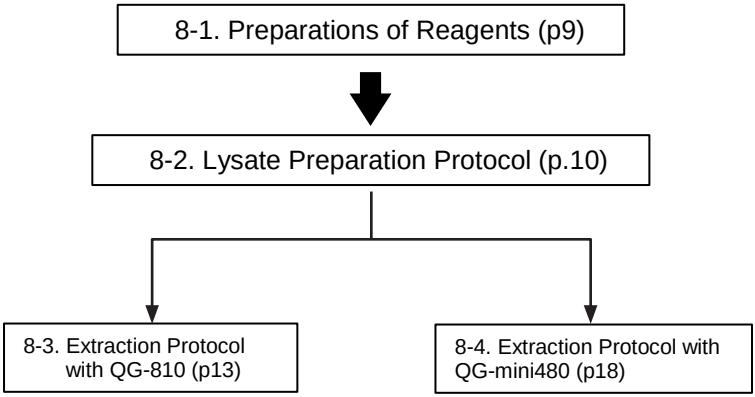
QuickGene RNA blood cell kit S (RB-S) corresponds to the extraction of total RNA from leucocytes (1.5×10^7 cells or less) after hemolysis. About 4,000-7,000 leucocytes are included in each µl of blood from healthy adults. Thus about 2 ml of the blood including 7,000 leukocytes/µl provides the maximum 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 leukocytes	Yield of total RNA recovered (µg)	A260/280
1×10^7	4.3	2

8. Protocol

〔Overview Flow Chart〕



8 - 1. Preparations of Reagents

◆LRB（75ml）

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.
0.5mol/L TCEP Solutions (Neutral pH) must be added to LRB before each use. Add 20 µl of 0.5mol/L TCEP Solutions (Neutral pH) per 1 ml of LRB. Use 520 µl of LRB per 1 Cartridge (CA2). Dispense in a fume hood and wear appropriate protective clothing.

◆CRB（100ml）

Make sure to use CRB for RNA elution.

◆DNase solutions (when using a DNase treatment)

Prepare according to the Details of Workflow (8-3<3> p.16, 8-4<3> p.21).

Use immediately after preparation.

◆WRB（280ml）

Provide the concentrated solution.
Add 120 ml of >99% ethanol into the bottle and mix with inversion the bottle gently at the beginning of use.
After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label.
Close the cap firmly to prevent volatilizing.

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

Prepare the required volume of WRB and CRB into the tubes (see Table 3): set them to Buffer Stand.

Table 3　Required volume of WRB and CRB

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

* Required volume of discharge

QG-810：WRB 8.0 ml, CRB 7.4 ml

Depending on the number of the Cartridges, add WRB and CRB.

Use 2.25 ml of WRB ml and 50 µl of CRB.

For example, in case of using 2 Cartridges, 12.5 ml of CRB and 7.5 ml of WRB are required.

* Use appropriate tubes according to Table1 (p.5).

8 - 2. Lysate Preparation Protocol

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

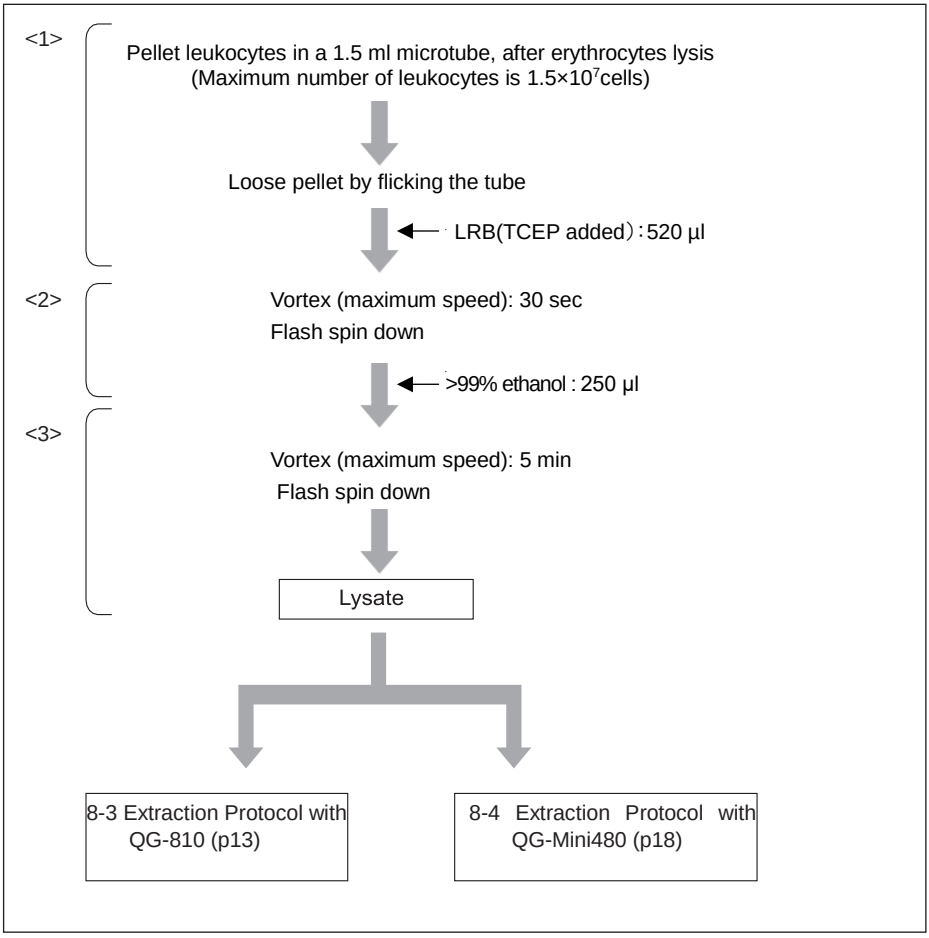
【Important notes before starting】

- 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 TCEP to LRB before use (p.9).

<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 (TCEP 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 (TCEP 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. (Complete the lysate.)

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.

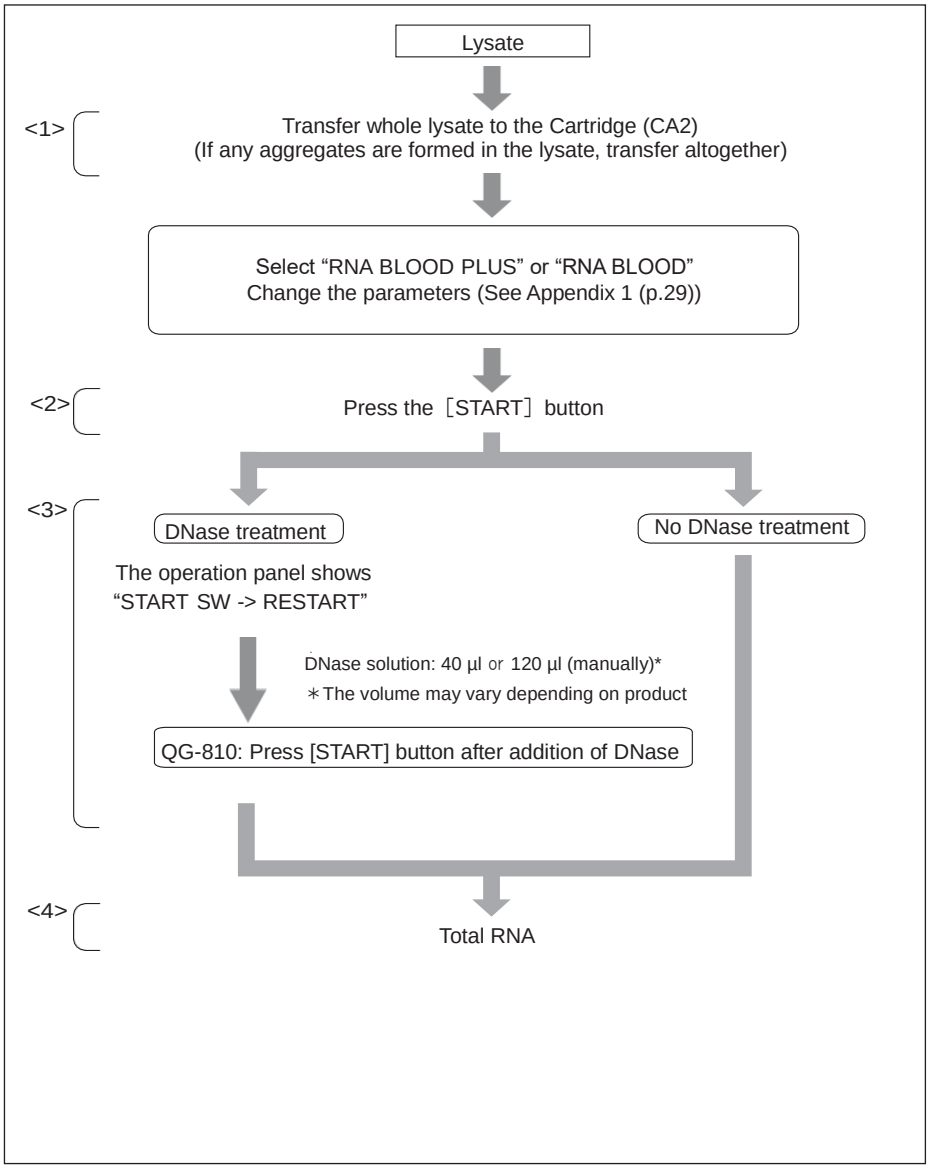
Perform the extraction operation quickly after completion of lysis.

8-3 Extraction Protocol with QG-810 (p.13)
8-4 Extraction Protocol with QG-Mini480 (p.18)

8 - 3. Extraction Protocol with QG-810

- Please read the User's Guide of QG-810 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 as the extraction mode for QG-810. (Appendix 1 p.29)
- 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 for the details of setting Cartridges (CA2), tubes and each reagent.
- Open the front cover of QG-810 and set the Collection Tubes (CT) and the 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 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. 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 Workflow



Details of QG-810 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). Close the front cover of QG-810 Confirm the appropriate mode on the operation panel and press the [START] button.
The operation panel shows “PROCESSING” (QG-810) 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
Extraction process	Stop
Extraction continuation	Possible*1

*1 QG-810: See User’s Guide of QG-810, “3.5 Operations to Restart Program from Pause” (p.29)

<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	Manufacturer	Cat.No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20U/40µl
DNase I, Amplification Grade	Thermo Fisher Scientific	18068-015		
RNase-Free DNase Set※1	QIAGEN	79254	2	3.4Kunitz units/40µl
DNase II, Amplification Grade	Sigma-Aldrich	AMP-D1	3	60U/120µl

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

Preparation 1)

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

Preparation 2)

2.7Kunitz 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)

1U/µl DNase II	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.

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)

<4> <Extraction completion> Operation panel displays the extraction results.

Table5 Extraction result

	QG-810	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 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.

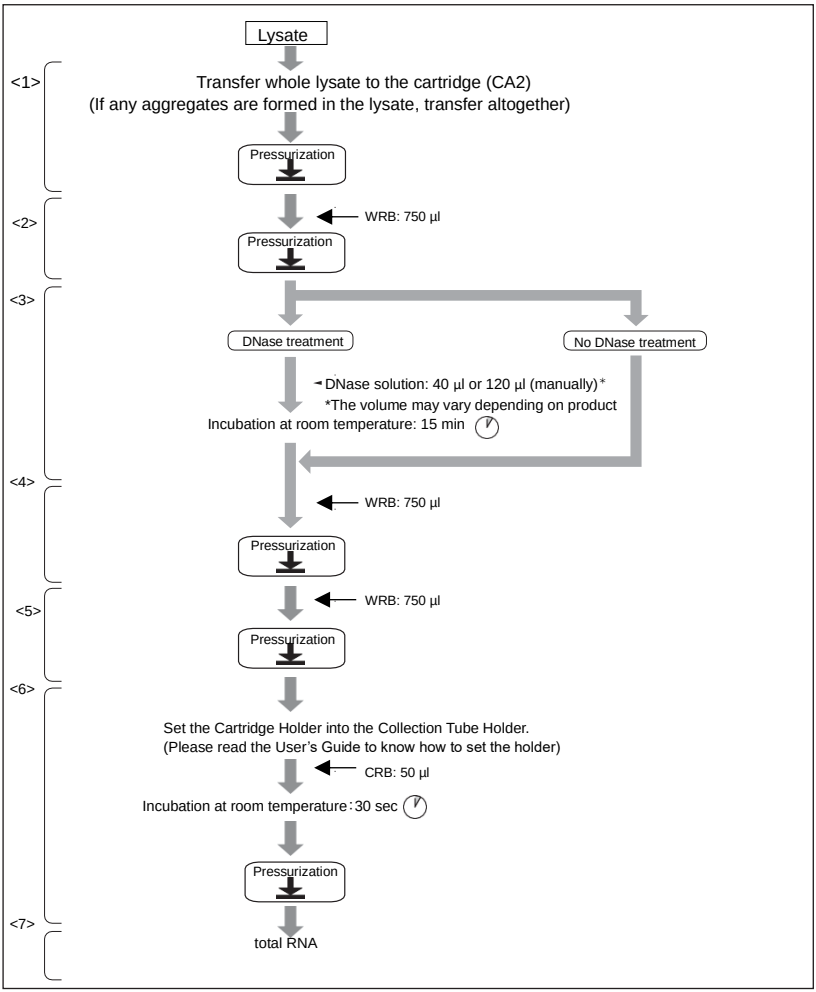
8 - 4. Extraction Protocol with QG-Mini480

- Please read the User's Guide of QG-Mini480 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 Waste Tube Holder.
- Set the Collection Tubes into the Collection Tube Holder in order. Cover it using Separator.
- Insert the Cartridge Holder into the correct position of the Waste Tube Holder. Make sure the bulges of Cartridge Holder are inserted into the side notches of the Waste Tube Holder. Keep the Holder Handle of the Collection Tube Holder toward the front side when setting the Collection Tubes as shown in below figure.
- Set the Pressure Seal Plate covering the Cartridges. Keep the stainless steel side up, the silicone rubber (Packing) side down and the Packing towards to the Cartridges while setting the Pressure Seal Plate. Check the position of Pressure Seal Plate to make sure the both ends of Pressure Seal Plate are inserted in the notches of Cartridge holder.
- Set the Cartridge Holder and Waste Tube Holder into QuickGene-Mini480, Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine the with a gentle push.
- Remove the Cartridge Holder from the Waste Tube Holder, and set it to the correct position of the Collection Tube Holder. Before setting the Cartridge Holder onto the Collection Tube Holder, make sure the Separator is covering on the Collection Tube Holder. Pull out the Separator. (See QuickGene-Mini480 Operation Manual：2 Operation)
- If after repeated compression fluid remains in any Cartridges (CA2), the Cartridges should be removed, and the steps shown in Troubleshooting ((3) p.24) taken.
- 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 of them according to the applicable regulations.

QG-Mini480 Workflow

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

1. Set the Cartridge Holder and the Waste Tube Holder in QG-Mini480.
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. Set the cartridge holder to the collection tube holder.
5. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
6. Pull out the Cartridge Holder and the Collection Tube Holder from QG-Mini480.



Details of QG-Mini480 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate prepared to 8-2 (p.10) to each Cartridge (CA2). Set the Pressure Seal Plate covering the Cartridges. Set the Cartridge Holder and Waste Tube Holder into QuickGene-Mini480. Keep the Holder Handle of the Waste Tube holder into QuickGene-Mini480. Make sure that the first row of Cartridge/Waste Tube Holder unit is placed at the stopper which located just under the Pressure Head inside the machine the with a gentle push. Make sure that no liquid remains in the Cartridges.

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. Pressure application automatically stops in about 70 seconds. If any lysate remains in the Cartridges even after about 70 seconds 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 Waste Tube Holder and then apply 750 µl of WRB to the Cartridges (CA2).Set the Cartridge Holder and the Waste Tube Holder into QG-Mini480.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 70 seconds. If any WRB remains in the Cartridges even after about 70 seconds 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> <DNase treatment> If not using a DNase, proceed to <4>. Prepare the DNase according to the following tables.

<3-1> Prepare the recommended DNase

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

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

Preparation 1)

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

Preparation 2)

2.7Kunitz 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)

1U/µl DNase II	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 Waste 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 Waste Tube Holder into QG-Mini480 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 Waste Tube Holder and then apply 750 µl of WRB to the Cartridges (CA2). Set the Cartridge Holder and the Waste Tube Holder into QG-Mini480. 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 70 seconds. If any WRB remains in the Cartridges even after about 70 seconds 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 Waste Tube Holder and then apply 750 µl of WRB to the Cartridges (CA2). Set the Cartridge Holder and the Waste Tube Holder into QG-Mini480. 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.

Do not add WRP three 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> Make sure the Separator is covering on the Collection Tube Holder. Pull out the Cartridge/Waste Tube Holder, Remove the Cartridge Holder from the Waste Tube Holder, and set it to the correct position of the Collection Tube Holder. Pull out the Separator. Insert the Cartridge Holder into the side notches of the Waste Tube Holder. (See QuickGene-Mini480 Operation Manual：2 Operation)
Apply 50 µl of CRB to the Cartridges (CA2) and then set the Cartridge Holder and the Collection Tube Holder in QG-Mini480. After 4 minutes of incubation at room temperature, 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 70 seconds. If any CRB remains in the Cartridges even after about 70 seconds 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 Collection Tube Holder. Pull the Cartridge Holder up from the Collection Tube Holder to correct position. Insert the Separator into the interval of the Cartridge and the Collection Tube. Remove the Cartridge Holder from the Collection Tube Holder, and then dispose of the Cartridges. Put Caps on the Collection Tubes and then remove them. Dispose of the Waste Tubes and waste solution 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) tightly, store at –20°C or –80°C.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA Blood kit S

(*): For QG-810

(**): For QG-Mini480

(1) Low yield or no Plasmid RNA obtained

Cause	Action
No addition of TCEP to LRB	Dispense a required volume of LRB before use, and add 20 µl of 0.5M TCEP solution (TCEP) 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 temperature.
Insufficient vortexing after addition of LRB (TCEP 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.9)
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: Confirm the parameters have been changed, particularly that the setting of the parameter of “ELUT VOL” (QG-810) or is correct (it should be “50”). In addition, in case air bubbles still remain in the line of QG-810, discharge them. For setting of parameter, refer to User’s Guide of QG-810. QG-Mini480: Confirm the amount of CRB is 50 µl.
Inadequate volume of CRB (*)	Confirm that the set volumes for each buffer to QG-810 are adequate.
Leaving Cartridges (CA2) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini480 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) set in QG-810 does not pass over 1 day.
No incubation performed at the time of elution	Confirm the parameters have been changed, particularly that the setting of the parameter of “ELUT DIP TM” (QG-810) is correct (it should be “30”). For setting of parameter, refer to User’s Guide of QG-810. QG-Mini480: 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.
RNA degradation	Refer to (3) “RNA degradation”
Temperature of operation is high	Take all of operation at room temperature (15-28°C)
Clogged filter (Operation panel: QG-810：－)	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 (TCEP added) or ethanol	After LRB or ethanol addition, perform vortexing completely for 30 sec or 5 min, respectively, according to section 8-2 (p.10). 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 leukocyte	Reduce the amount of leukocytes.
Inadequate lysis of leukocytes	After LRB (TCEP added) 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-Mini480 has been started, work quickly without interruption.
QG-810: Operation panel of " – (QG-810)" is displayed, and lysate or WRB is remained even after pressurization (*) QG-Mini480: 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.9).

(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 TCEP to LRB	Dispense a required volume of LRB before use, and add 20 µl of TCEP 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). 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: Confirm that the parameter “WASH VOL 1-5” and “WAS2 VOL 1-5” is “750” ; refer to Appendix 1 (p.29). QG-Mini480: 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-Mini480: 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) (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.
No addition of CRB (**)	Add 50 µl of CRB after the transfer of Cartridge Holder to the Elution Position or the transfer of the Cartridge Holder from the Waste Tube Holder to the Collection Tube Holder.
No transfer of the Cartridge Holder to the Elution Position when adding CRB or No transfer of the Cartridge Holder from the Waste Tube Holder to the Collection Tube Holder (**).	When adding CRB, addition has to be started after the transfer of Cartridge Holder to the Elution Position or the transfer of the Cartridge Holder from the Waste Tube Holder to the Collection Tube Holder.

(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:

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-Mini480:

<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-Mini480 toward the front side to begin pressurization. After checking that no lysate remains in the Cartridge, perform the operation from 8-4 <2> (p.20).
If complete removal of DNA is required, include a DNase treatment as per normal after the first wash step.

<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-Mini480 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-Mini480 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	Parameter	
		RNA BLOOD PLUS (With DNase Treatment)	RNA BLOOD (Without DNase Treatment)
1	BIND PEAK	120	120
2	WASH COUNT	1	3
3	WASH PEAK	110	110
4	WASH VOL1	750	750
5	WASH VOL2	750	750
6	WASH VOL3	750	750
7	WASH VOL4	750	750
8	WASH VOL5	750	750
9	WASH DIP TM	150	150
10	WAS2 WAIT T	15	0
11	WAS2 COUNT	2	0
12	WAS2 PEAK	110	110
13	WAS2 VOL1	750	750
14	WAS2 VOL2	750	750
15	WAS2 VOL3	750	750
16	WAS2 VOL4	750	750
17	WAS2 VOL5	750	750
18	ELUT VOL	50	50
19	ELUT PEAK	100	100
20	ELUT DIP TM	30	30

<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 value 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: raise 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 Erythrocyte Lysis

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

Hemolytic agent (HB)

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

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 x 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 2,000 x 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 step1. 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 x 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.

* Trademark and exclusion item
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RB-S_HB-J_V4.2