KURABO

HANDBOOK

QuickGene RNA cultured cell kit S (RC-S)

For extraction of total RNA from cultured cells

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 cultured cells. 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 17 min (without DNase treatment)
QuickGene-Mini80 (QG-Mini80) : about 16 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	LRC	75 ml
☐ Wash Buffer	WRC	210 ml
☐ Elution Buffer	CRC	100 ml
☐ Cartridges	CA	96
☐ Collection Tubes	CT	96
☐ Caps	CAP	96
☐ Waste Tubes	WT	96

2-2 Storage Conditions

All reagents are stable for 9 months after purchase at room temperature (15-28°C).

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

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

* Prepare if necessary

- Sterilized PBS (for resuspension of frozen pelleted cells)
- 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)
  - DNase I, Amplification Grade (Sigma-Aldrich : Cat. No. AMP-D1)
  - Deoxyribonuclease (RT Grade) (Nippon Gene : Cat. No. 313-03161)
  - DNase I, RNase-Free (Life Technologies : Cat. No. AM2222)
  - RNase-Free DNase Set (QIAGEN : Cat. No. 79254)

[2] Equipments

- QuickGene
- Centrifuge tubes* (Large/Small sets)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- Tube stand
- Vortex mixer (maximum speed 2,500 rpm or more)
- Benchtop microcentrifuge (c.a. 5,000 rpm)

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

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

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

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

◆ LRC (Lysis Buffer)
- **Harmful if ingested.**
- Do not drink or ingest. Avoid contact with eyes.
- It should be handled at a well-ventilated place. Wear 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.

◆ WRC (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.

◆ CRC (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 LRC at high temperature should be avoided.

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

◆ **In the case of using a 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

- This kit is applicable to cultured cells up to the upper limit of 1×10^6 cells per each treatment. In case addition is performed from more number of cells than the prescribed one, clogging and/or decrease in yield may be resulted from.
- In case clogging occurs, try by reducing the number of cells.
- The yield varies depending upon sample conditions (cell type, culture condition, stage of growth, etc.).

◆ Use of Reagent

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

◆ Procedure of Extraction

- Use QuickGene RNA cultured cell kit S (RC-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.12), Appendix 1 (p.30) and Appendix 2 (p.31)
  QG-Mini80 : 8-4 (p.17)
- Refer to QuickGene User's Guide for details.

<Prevention Against RNase Contamination>

- Wear disposable gloves when you are handing 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.

◆ Other Precautions

- Tailing might be caused on high molecular weight in non-denaturing agarose gel analysis. There is no problem on the quality of total RNA.

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene RNA cultured cell kit S (RC-S) is evaluated routinely on a lot-to-lot uniformity.
- QuickGene RNA cultured cell kit S (RC-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

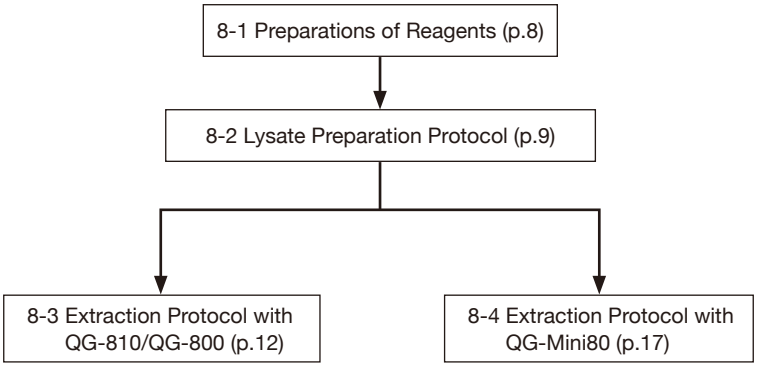
QuickGene RNA cultured cell kit S (RC-S) corresponds to the extraction of total RNA from cultured cells (1×10^6 cells or less). Yield and purity (A260/280, A260/230) of total RNA extracted from 1×10^6 cultured cells are shown in Table 2.

Table 2 Example of the extraction of total RNA from 1×10^6 cells of HL60 with this kit

	DNase (+)	DNase (-)
Yield (μg)	9.7	11.7
A260/280	1.94	1.90
A260/230	2.08	2.18

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

◆ LRC (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 LRC before each use. Add 10 µl 2-ME per 1 ml of LRC. Dispense in a fume hood and wear appropriate protective clothing.

◆ WRC (210 ml)

WRC is supplied as a concentrate.
Add 90 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.

◆ CRC (100 ml)

Use CRC for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to the Details of Workflow (<3> p.15, 20).
Use immediately after preparation.

◆ Required volume of WRC (>99% ethanol added) and CRC (in the case of using a QG-810/QG-800)

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

Table 3 Required volume of WRC and CRC

Number of Cartridges	WRC (QG-810/QG-800)	CRC (QG-810)	CRC (QG-800)
8	20 ml	9 ml	8 ml
16	32 ml	11 ml	11 ml
24	44 ml	13 ml	13 ml
32	56 ml	15 ml	15 ml
40	69 ml	17 ml	17 ml
48	81 ml	19 ml	19 ml
56	93 ml	21 ml	21 ml
64	106 ml	22 ml	22 ml
72	118 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WRC 8.0 ml, CRC 7.4 ml
QG-800 : WRC 8.0 ml, CRC 6.4 ml
Depending on the number of the Cartridges, add WRC and CRC.
Use WRC 1.5 ml and CRC 100 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 11 ml of WRC, 7.6 ml of CRC (QG-810) and 6.6 ml of CRC (QG-800) are required.
*Use appropriate tubes according to Table 1 (p.4).

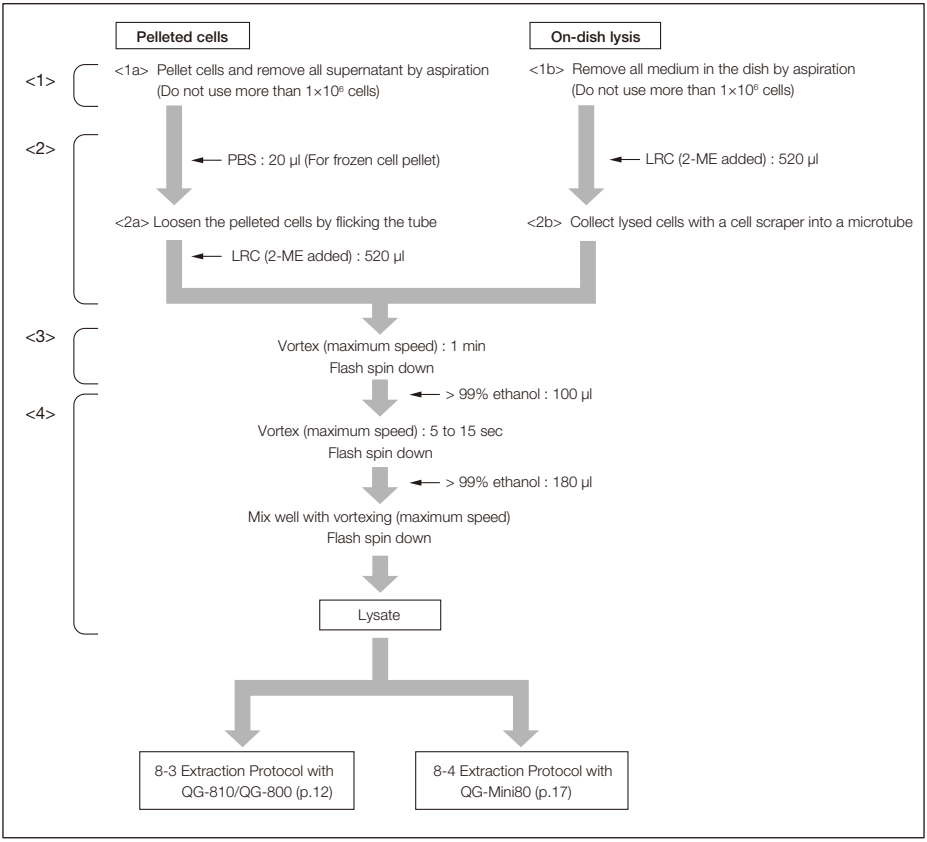
8-2 Lysate Preparation Protocol

QuickGene RNA cultured cell kit S (RC-S) corresponds to the extraction of total RNA from cultured cells (1×10^6 cells or less).

[Important notes before starting]
- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow (p.10).
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- Any solution and waste fluid containing LRC should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

[Preparations for starting the experiment]
- WRC is supplied as a concentrate. Check that 90 ml of >99% ethanol is added to WRC before starting an experiment.

Lysate Preparation Workflow



Details of Lysate Preparation Workflow

<1> Count the number of cells exactly.

If the number of cells is too many, it may cause significant decrease in the yield or precision of extraction, or sometimes clogging. When clogging occurs, reduce the number of cells, and then try again.

<1a> To lyse pelleted cells :

- To make pellet from adherent cells :
  Please collect, and count the cells by the trypsin processing. Centrifuge cells for 5 min at 300 × g, and aspirate supernatant.
- To make pellet from cells grown in suspension :
  Pellet cells for 5 min at 300 × g in a tube. Carefully remove all supernatant, and wash the pelleted cells with PBS. Centrifuge it at 300 × g for 5 min again. Carefully remove supernatant.

  Pelleted cells can be stored at –70°C if it is frozen with liquid nitrogen. Please make sure to count the number of cells before freezing.

<1b> To lyse cells directly in culture dish (On-dish lysis) :
Remove the all of medium by aspirator.

<2> Add LRC to lyse cells.
Add 2-ME to LRC before use (p.8).

<2a> To lyse pelleted cells :
Loosen cells by flicking the microtube and then add 520 µl of LRC.
When a frozen pellet is used, add about 20 µl of PBS to the pelleted cells, resuspend cells by flicking, and then add LRC.

<2b> To lyse cells directly in cultured dish (On-dish lysis) :
Add 520 µl of LRC to a flask or a dish, mix cells and LRC well with the aid of a cell scraper or the like, and transfer the whole lysed cells to a microtube.

<3> Vortex at the maximum speed for 1min. Flash spin down for several seconds to remove drops from the inside of the lid.

<4> Add 100 µl of >99% ethanol, and mix adequately with vortexing (about 5 to 15 sec). Flash spin down for several seconds to remove drops from the inside of the lid. Further, add 180 µl of ethanol, and mix adequately with vortexing. Flash spin down for several seconds to remove drops from the inside of the lid.

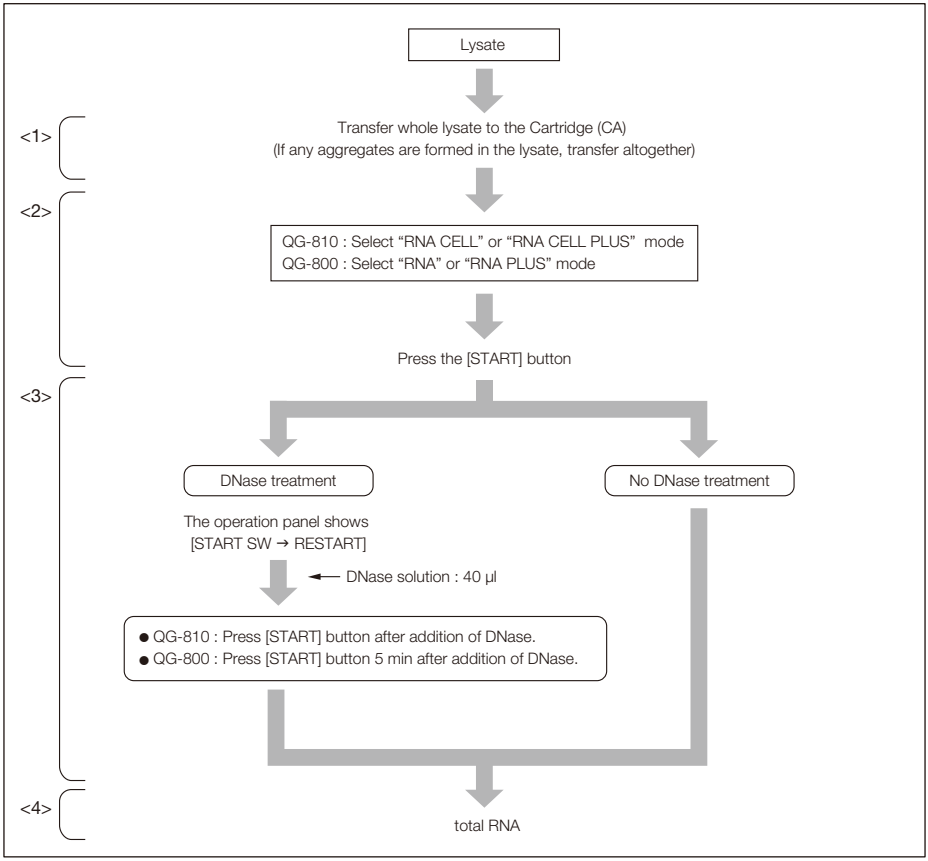
Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.12)
QG-Mini80 (p.17)

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 90 ml of >99% ethanol is added to WRC before starting an experiment.
- Select the "RNA CELL" or "RNA CELL PLUS" mode as the extraction mode for QG-810.
- Select the "RNA" or "RNA PLUS" mode as the extraction mode for QG-800.
- All reagent, Cartridges and tubes are manufactured in clean rooms. Wear gloves during the experiments to avoid nuclease contamination.
- Refer to the User's Guide of QG-810/QG-800 for the details of setting Cartridges (CA), tubes and each reagents.
- 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 (CA).
- Set WRC (>99% ethanol added) and CRC to QG-810/QG-800 referring to p.8.
- Incorrect Cartridge (CA) placement may result in the solution spilling or improper extraction.
- Press the [DISCHARGE] button after closed the front cover of QG-810/QG-800. Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- Any solution and waste fluid containing LRC should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose of 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.9) to the each Cartridge (CA).

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.30), 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 step <4>. Preapare 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	20U/40 µl
DNase I, Amplification Grade	Life Technologies	18068-015		
DNase I, Amplification Grade	Sigma-Aldrich	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Life Technologies	AM2222	2	40U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4Kunitz units/40 µl

*1 : Dissolve 1,500Kunitz 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 U/µl DNase I	20 µl
10 × Reaction Buffer	4 µl
Nuclease-Free water	16 µl

Preparation 3)

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.

<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 (CA).
Add 40 µl of any DNases 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 5 min (the operation panel shows “PROCESSING”). Default waiting (holding) time of DNase treatment is 5 min. You can change the setting of time as the parameter of a program. (Parameter of “WAS2 WAIT T” see Appendix1, p.30)

<3-2b> For QG-800
Close the front cover, incubate at room temperature on the Cartridge (CA) for 5 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 will be 100 µl.
Cover with 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 (CA).
Dispose the fluid in the Discharge Tray also.

8-4 Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 90 ml of >99% ethanol is added to WRC before starting an experiment.
- Set Waste Tubes (WT) into the Tube Holder.
- Set Tube Adapters to the Tube Holder, and set Collection Tubes (CT). In substitution for Collection Tubes, you can use 1.5 ml microtubes. In this case Tube Adapters are unnecessary.
- Insert the Cartridge Holder into the Wash Position (W) of the Tube Holder. Then set Cartridges (CA) to the Cartridge Holder. Confirm the Release Lever is positioned at the left-hand end of the Cartridge Holder.

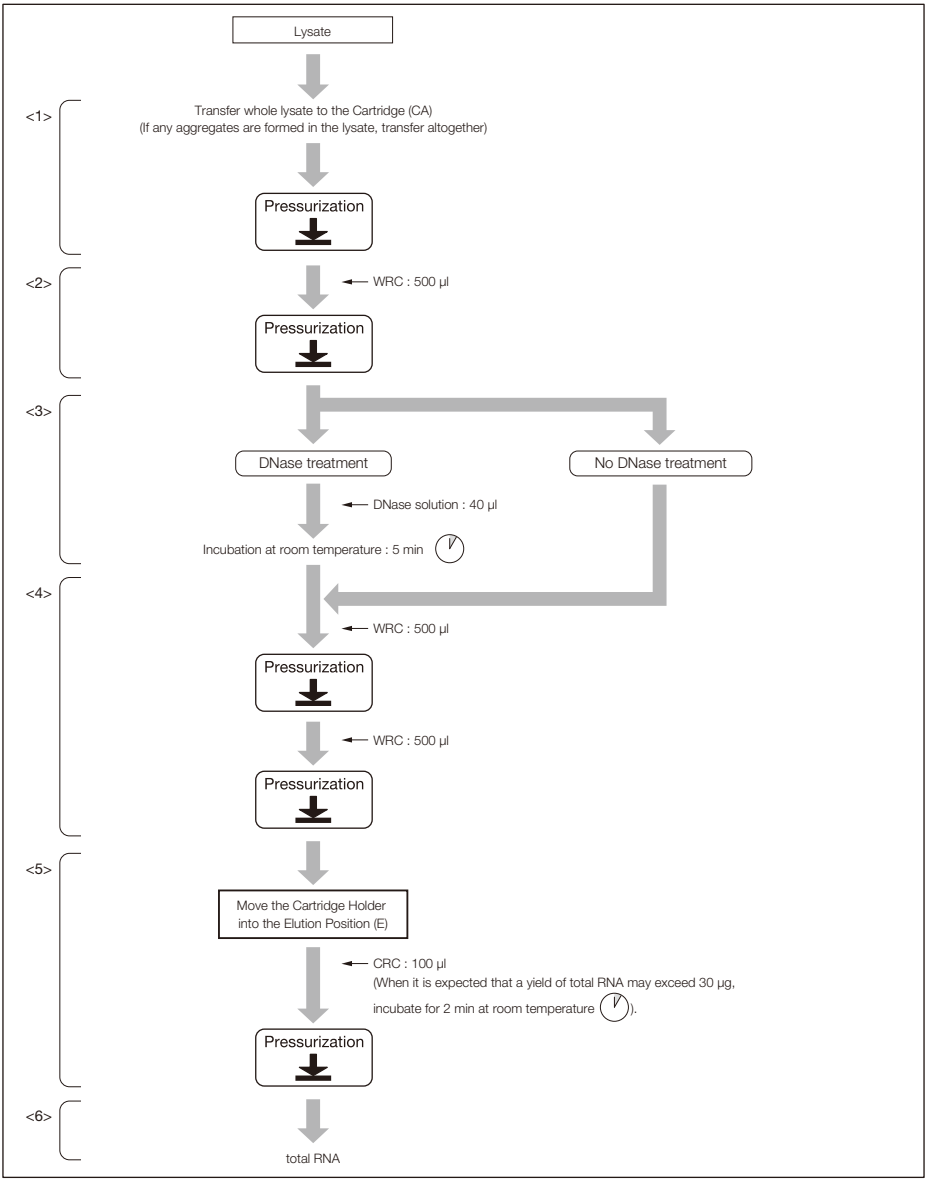
- When setting the Tube Holder and the Cartridge Holder to QG-Mini80, insert until to the end.
- When pressuring lysates and WRC (>99% ethanol added), confirm that Wash Label on the Tray can be entirely seen.
- When pressuring CRC, confirm that Wash Label on the Tray can not be seen, being hidden below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

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

QG-Mini80 Workflow

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

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



Details of QG-Mini80 Workflow

<1> <Applying Lysate> Carefully transfer the whole lysate prepared at 8-2 (p.9) to each Cartridge (CA). Set the Tube Holder and Cartridge 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 500 µl of WRC to the Cartridges (CA). Set the Tube Holder and the Cartridge 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 WRC remains in the Cartridges and then return the Rotary Switch to the original position.

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

<3> <DNase treatment> If not using a DNase, proceed to step <4>. Preapare 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	20U/40 µl
DNase I, Amplification Grade	Life Technologies	18068-015		
DNase I, Amplification Grade	Sigma-Aldrich	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Life Technologies	AM2222	2	40U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4Kunitz units/40 µl

*1 : Dissolve 1,500Kunitz 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 U/µl DNase I	20 µl
10 × Reaction Buffer	4 µl
Nuclease-Free water	16 µl

Preparation 3)

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.

<3-2> Method of DNase treatment on column

Pull out the Tube Holder and the Cartridge Holder, add 40 µl of any DNases per Cartridge. After addition, set the Tube Holder and the Cartridge Holder to QG-Mini80, incubate at room temperature for 5 min.

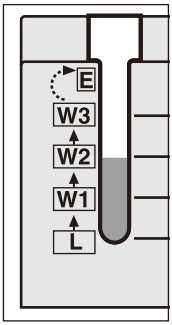
Avoid touching the filter in the Cartridge with the pipette tip during addition of DNase solution.
Pressurization should not be performed during incubation.
It is necessary to start pressurization after addition of WRC for the second wash (<4>).
It is necessary to start DNase treatment after the first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

<4> <Second wash> Pull out the Cartridge Holder and the Tube Holder and then apply 500 µl of WRC to the Cartridges (CA). Set the Tube Holder and the Cartridge 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 WRC remains in the Cartridges and then return the Rotary Switch to the original position.

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

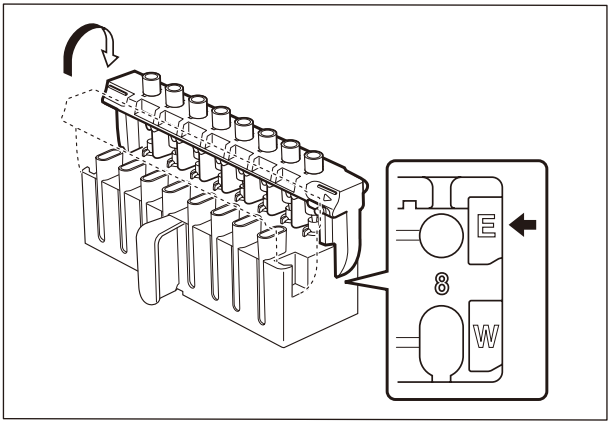
<5> <Third wash> Pull out the Cartridge Holder and the Tube Holder and then apply 500 µl of WRC to the Cartridges (CA). Set the Tube Holder and the Cartridge Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WRC remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WRC 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 [W2], not [W3] position. (Refer to the following illustration)
Do not add WRC 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 100 µl of CRC to the Cartridges (CA) and then set the Cartridge Holder and Tube Holder in QG-Mini80. After setting it, check the Wash Label cannot be seen by hiding under the Tube Holder.
When you perform DNase treatment and the yield of total RNA may exceed 30 µg, incubate the Cartridge for 2 min after applying CRC.
Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no CRC remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CRC 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 Tube Holder and the Cartridge Holder. Remove the Cartridge Holder from the Tube Holder and then dispose of the Cartridges (CA). When sliding the Release Lever to the right-hand end, all Cartridges will fall down. Remove the Collection Tubes and then insert the Collection Tubes into the Tube Rack (optional) and then put Caps (CAP).
When the Tube Rack is not used, remove the Collection Tubes after putting Caps on them.
When using commercially available 1.5 ml microtubes : Put caps on 1.5 ml microtubes and then remove them.
Dispose of 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 cultured cell kit S (RC-S).

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

(1) Low yield or no RNA obtained :

Cause	Action
Inadequate removal of medium from flask or dish	Remaining medium causes dilution of LRC, possibly leading to decrease in the yield. Remove all medium from flask or dish.
Inappropriate number of cells	Count the number of cells, and perform extraction within an appropriate range of the number of cells.
No addition of 2-ME to LRC	Dispense a required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRC.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, add 20 µl of PBS after cells are thawed, flick adequately to completely loosen pellet.
Formation of a precipitate in LRC	Check before use that there is no precipitate formed. In case a precipitate is formed, warm to 37°C to dissolve the precipitate, and use the resulting solution after cooling back to room temperature.
Insufficient homogenization after addition of LRC	Vortex at the maximum speed for 1 min.
No addition of the prescribed volume of ethanol to WRC	Before using WRC for the first time, ensure that the prescribed volume of >99% ethanol has been added.
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Insufficient volume of CRC	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of “CLCT VOL” (QG-800) or “ELUT VOL” (QG-810) is correct (it should be “100”). 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 CRC is 100 µl.
Inadequate volume of any buffer (*)	Confirm that the set volumes for each buffer to QG-810/QG-800 are adequate.
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 WRC to the Cartridge (CA) after the 5 min incubation following the addition of a DNase solution (**)	Add a DNase solution, incubate for 5 min, then add WRC to the Cartridge before pressurization.
Rupturing of filter when adding a DNase (When using a DNase)	Be careful not to allow pipette tip to contact with a filter in the Cartridge (CA).
Not taking 2 min incubation time after addition of CRC (**)	When you perform DNase treatment and the yield of total RNA may exceed 30 µg, incubate the Cartridge (CA) for 2 min after applying CRC.
Shortage of incubation time after applying CRC (**)	Although no incubation time is specified for CRC in the present protocol except for the cases you perform DNase treatment and the yield of total RNA may exceed 30 µg, incubate the Cartridge (CA) for 2 min after applying CRC. Prolongation of incubation time period of CRC may sometimes result in increase in the yield.

Cause	Action
Addition of excessive volume of CRC (**)	Reducing a liquid volume of CRC can increase RNA concentration. Nevertheless, the yield of RNA is decreased. This decrease in the yield may sometimes be reduced by prolongation of incubation time period of CRC.
RNA degradation	Refer to (4) “RNA degradation”.
Temperature of operation is high	Take all of operation at room temperature (15-28°C).

(2) Purity of RNA is low :

Cause	Action
Formation of foams when applying lysate	Foams produced at the time of applying lysate last to the end, causing reduction in a yield of a total RNA. Avoid the formation of foams at the time of adding any lysate.
Improper washing procedure (**)	Wash three times with 500 µl of WRC.
Use of reagents other than CRC to elute RNA	Use CRC to elute RNA.
Use of too much amount of cells	Reduce the number of cells.

(3) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of cells	Reduce the number of cells.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, add 20 µl of PBS after cells are thawed, flick adequately to completely loosen pellet.
No addition of the prescribed volume of ethanol to WRC	Before using WRC, for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.8.)
Insufficient homogenization after addition of LRC	Vortex at the maximum speed for 1 min.
Insufficient homogenization after addition of ethanol	After addition of prescribed volume of >99% ethanol, vortex sufficiently (for 1 min).
Inadequate vortexing	It is recommended to vortex for 1 min after addition of LRC, whereas there are some cases where the problem of clogging is improved by extending vortexing longer. Vortexing after addition of ethanol exhibits the same effects. However, decrease of the yield of RNA may occur in some cases.
Insufficient pressurization (**)	Pressurize once more.
QG-810/QG-800 : Operation panel of “- (QG-810)” or “× (QG-800)” is displayed, and lysate or WRC is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRC completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA), and try the recovery of RNA according to p.27.

(4) RNA degradation :

Cause	Action
No addition of 2-ME to LRC	Dispense a required volume of LRC before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRC.
RNase contamination	Although all buffers, Cartridges (CA), and Collection Tubes (CT) 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 DNase)	Use any one of the recommended RNase-free DNases. For details, inquire to each maker.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(5) 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 CELL PLUS” (QG-810) or “RNA PLUS” (QG-800). Refer to the following (6) when the degradation of DNA is insufficient.
RNA degradation	Refer to (4) “RNA degradation”.
Improper washing procedure (**)	Wash three times with 500 µl of WRC.

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

Cause	Action
Filter was not completely soaked in DNase solution	Make sure that DNase solution is evenly covered over the filter in the Cartridge (CA) when DNase solution is added.
Insufficient DNase activity	Use a recommended DNase activity.
Insufficient incubation time for DNase treatment	In the case of QG-800 or QG-Mini80, incubate at room temperature (15-28°C) for 5 min. In the case of QG-810, confirm that the parameter “WAS2 WAIT T” is “5”.
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.

(7) 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.

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

Cause	Action
Insufficient set of CRC or no operation of discharging (*)	Set the prescribed volume of CRC 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 CRC (**)	After insert the Cartridge Holder to the Elution Position (E), add 100 µl of CRC to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CRC (**)	When adding CRC, addition has to be started after the transfer of Cartridge Holder to the Elution Position (E).

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

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

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

In case of QG-810/QG-800

a) If lysate remain in Cartridge (CA) :
   Transfer the lysate remaining in the Cartridge (CA) to a new Cartridge, and perform the operations from 8-3 <1> (p.13) again.
   For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If WRC remain in Cartridge (CA) :
   For the recovery of RNA from a clogged filter in the Cartridge, proceed step 3) prescribed below.

In case of QG-Mini80

a) If clogging occurs at the lysate pressurization step :
   Transfer the lysate remaining in the Cartridge (CA) to a new Cartridge, and perform the operations from 8-4 <1> (p.19) again.
   For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If clogging occurs at the washing step :
   For the recovery of RNA from a clogged filter in the Cartridge, proceed step 3) prescribed below.

[Method for Recovering RNA from Clogged Cartridge (CA)]

Start from 1) in case of a), from 3) in case of b)

Preparation : tip-curved acuminate tweezers or tweezers for otolaryngology use

1) Discard the lysate remaining in a Cartridge(CA).
2) Add 500 µl of WRC to the Cartridge, and pipette it gently several times.
3) Discard WRC in the Cartridge.
4) Dispense 200 µl of CRC to a 1.5 ml microtube in advance.
5) Prepare tweezers with finely tapered, curved tips or an otolaryngologic tweezers. By roasting the tip of the tweezers with a burner flame or wiping with an RNase remover to ensure that they are not contaminated with RNase.
6) According to Figures 1 and 2, dismount filter from Cartridge (CA) with pushing rim of filter with the tip of tweezers.
7) Soak the dismounted filter into CRC placed in 1.5 ml microtube, and incubate at 65°C for 10 min.
8) Flash spin down for several seconds to remove the drops from inside of the lid.
9) Transfer the solution from 8) to another 1.5 ml microtube, and add 320 µl of LRC.
10) Vortex at the maximum speed for 1 min then flash spin down.
11) Add 100 µl of >99% ethanol.
12) Vortex at the maximum speed for 10 sec then flash spin down.
13) Add 180 µl of >99% ethanol.
14) Vortex at the maximum speed for 5 sec then flash spin down.
15) Add the whole volume of the lysate prepared by the treatment at 14) to a Cartridge (CA), and perform the following operations.
    Operations from 8-3 <1> (p.13) or 8-4 <1> (p.19).

Figure 1 Appearance of a tweezers put into Cartridge (CA)

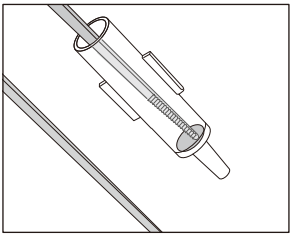
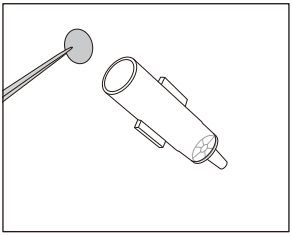


Figure 2 Appearance of a filter dismounted



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 a QG-810 select “RNA CELL PLUS” or “RNA CELL” mode, the parameter of “RNA CELL” or “RNA CELL PLUS” is the following Table.

Display Sequence	LCD message	Parameter	
		RNA CELL PLUS (With DNase Treatment)	RNA CELL (Without DNase Treatment)
1	BIND PEAK	120	120
2	WASH COUNT	1	3
3	WASH PEAK	110	110
4	WASH VOL1	500	500
5	WASH VOL2	500	500
6	WASH VOL3	500	500
7	WASH VOL4	500	500
8	WASH VOL5	500	500
9	WASH DIP TM	150	150
10	WAS2 WAIT T	5	0
11	WAS2 COUNT	2	0
12	WAS2 PEAK	110	110
13	WAS2 VOL1	500	500
14	WAS2 VOL2	500	500
15	WAS2 VOL3	500	500
16	WAS2 VOL4	500	500
17	WAS2 VOL5	500	500
18	ELUT VOL	100	100
19	ELUT PEAK	100	100
20	ELUT DIP TM	30	30

When changing the parameter, refer to QG-810 User’s Guide.

Appendix 2 Setting of QG-800 Parameter

In the case of using a QG-800 select “RNA PLUS” or “RNA” mode, the parameter of “RNA” or “RNA PLUS” is the following Table.

Display Sequence	Operation menu	Parameter	
		RNA PLUS (With DNase Treatment)	RNA (Without DNase Treatment)
1	SMAP PEAK	120	120
2	WASH COUNT	1	3
3	WASH PEAK	110	110
4	WASH VOL1	500	500
5	WASH VOL2	500	500
6	WASH VOL3	500	500
7	WASH VOL4	500	500
8	WASH VOL5	500	500
9	WAS2 COUNT	2	0
10	WAS2 PEAK	110	110
11	WAS2 VOL1	500	500
12	WAS2 VOL2	500	500
13	WAS2 VOL3	500	500
14	WAS2 VOL4	500	500
15	WAS2 VOL5	500	500
16	CLCT VOL	100	100
17	CLCT PEAK	120	120

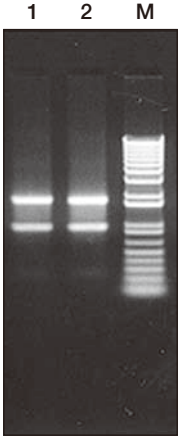
When changing the parameter, refer to QG-800 User’s Guide.

Appendix 3 Examples of the Data with QuickGene RNA cultured cell kit S (RC-S)

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

Figure 3 illustrates the result of electrophoresis of total RNA extracted with this kit.

Figure 3



No.	Sample
1	HeLa (1 well/6-well Plate)
2	

M : Maker (1 Kb DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE Buffer

● **Yield and purity (A260/280, A260/230)**

The yield and purity of total RNA extracted with this kit as following Table.

	Number of cells	Yield (μg)	Purity	
			A260/280	A260/230
HL60	1.0×10^6	9.7	1.88	2.08
NIH/3T3	0.3×10^6	15.6	2.17	2.18
COS-7	0.8×10^6	34.4	1.96	2.17
HeLa	1.2×10^6	28.1	2.28	2.21
HEK293	2.1×10^6	30.4	2.27	2.14

● **RT-PCR**

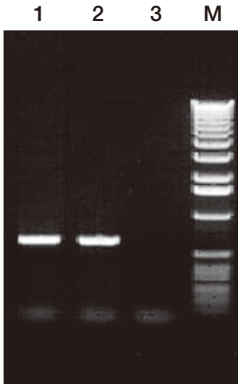
Figure 4 illustrates that the result of RT-PCR amplification, which performed using diluted total RNA extracted with this kit.

RT-PCR was performed with G3PDH mRNA at following condition.

<RT reaction condition>
Template : HL60 total RNA 500 ng
Enzyme : SuperScript II (Invitrogen)

<PCR condition>
Template : cDNA (10 pg/µl total RNA)
Primer : G3PDH Primer
Enzyme : Takara Taq Start Version

Figure 4



No.	Sample
1	HeLa (1 well/6-well Plate)
2	Positive Control
3	Negative Control

M : Marker (100 bp DNA Ladder : Invitrogen)
Electrophoresis condition : 1% Agarose/1 × TAE Buffer

RT-PCR amplification was performed successfully using 10 pg/µl total RNA.

KURABO

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RC-S_HB-E_V30