KURABO

HANDBOOK

QuickGene RNA cultured cell HC kit S (RC-S2)

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. This kit is designed for extraction of total RNA from the number of cells corresponding to 6 to 10 cm dish. 3 to 15 × 10^6 cells grown in suspension can be used. No hazardous organic solvents such as phenol and chloroform are used. RNA from 8 sets (when using 6 cm dish), or 4 sets (when using 10 cm dish) of cell lysate samples can be simultaneously extracted in following time.
QuickGene-810/QuickGene-800 (QG-810/QG-800) : about 15 min (without DNase treatment)
QuickGene-Mini80 (QG-Mini80) : about 14 min (without DNase treatment)
The purified, high quality total RNA is suitable for RT-PCR, Northern blot, microarray 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)

The kit contains sufficient reagents for 48 total RNA extractions from 10 cm dishes or 96 extractions from 6 cm dishes.

☐ Lysis Buffer	LRP	85 ml
☐ Solubilization Buffer	SRP	40 ml
☐ Wash Buffer	WRP	360 ml
☐ Elution Buffer	CRP	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 LRP)
- >99% Ethanol (for preparation of lysate and WRP working solution)

* Prepare if necessary

- DNase [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
- Ball mill homogenizer (TOMY Micro Smash MS-100/QIAGEN TissueLyser, etc.)
- Centrifuge tubes*1 (Large/Small sets)
- Tubes appropriate for ball mill homogenizer
  TOMY Micro Smash MS-100 : TOMY 2 ml tube (Cat. No.TM-625)*2
  QIAGEN TissueLyser : TreffLab 2.0 ml click cap (Cat. No. 96.9329.9.01)
- Ball : zirconia, 5mmϕ for TOMY MS-100
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Benchtop microcentrifuge

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

*2 Sterilized tube is not recommended. Tube may be broken.

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 WRP)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRP)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRP)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRP)	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/)

◆ LRP (Lysis Buffer)
- **Harmful if ingested.**
- Do not drink or ingest. Avoid contact with eyes.
- It should be handled in 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.

◆ SRP (Solubilization 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.

◆ WRP (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.

◆ CRP (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 LRP at high temperature should be avoided.

◆ Any solution and waste fluid containing LRP 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

- This kit is applicable to an upper limit of cells equivalent to the number of confluent cells on a 10 cm dish. It is necessary to count the number of cells, and check that it is within the range indicated in Table 4 (p.11) before starting the extraction.
- Strictly follow the instructions Table 4 (p.11) for the amount of sample to be added to each Cartridge (CA) described in the respective protocol (p.11 to 27). Do not overload the Cartridge, as this will significantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- In case of 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 LRP during storage, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.

◆ Procedure of Extraction

- Use QuickGene RNA cultured cell HC kit S (RC-S2) at room temperature (15-28°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- During the procedure, work quickly without interruption.
- During homogenization, tube rupture may sometimes occur when the centrifuge is operated beyond the prescribed speed or when microtubes or balls other than the prescribed ones are used. It is necessary to use a homogenizer at prescribed speed, and use a prescribed microtube and ball.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : 8-3 (p.18), Appendix 1 (p.35) and Appendix 2 (p.37)
  QG-Mini80 : 8-4 (p.22)
- Refer to QuickGene User's Guide for the 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.

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene RNA cultured cell HC kit S (RC-S2) is evaluated routinely on a lot-to-lot uniformity.
- QuickGene RNA cultured cell HC kit S (RC-S2) 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 HC kit S (RC-S2) corresponds to the extraction of total RNA from cultured cells. Examples of total RNA recovered are shown in Table 2.

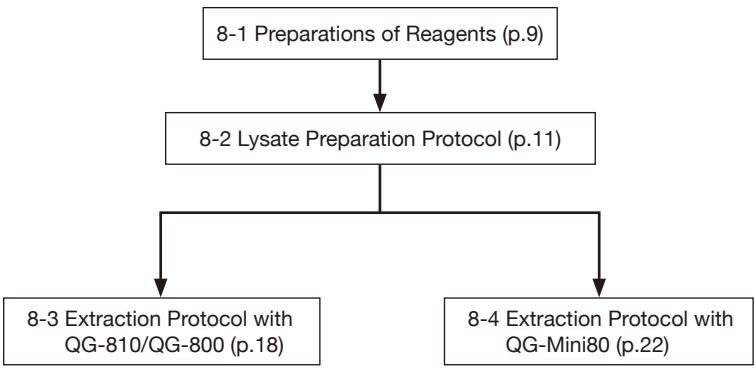
Table 2 Examples of yield of total RNA extracted from various model cells
(Homogenized with a TOMY Micro Smash MS-100 ; with DNase treatment)

Dish size	Adherent cells ( ) is yields from pelleted cells								Non-adherent cells	
	HeLa		HEK293		COS-7		NIH/3T3		HL60	
	Number of cells ($\times 10^6$ cells)	Yield (µg)	Number of cells ($\times 10^6$ cells)	Yield (µg)	Number of cells ($\times 10^6$ cells)	Yield (µg)	Number of cells ($\times 10^6$ cells)	Yield (µg)	Number of cells ($\times 10^6$ cells)	Yield (µg)
6cm	1.5-3.5	45-70	3.0-5.0	40-90	0.5-1.5	20-40	1.0-2.5	25-30	3.0-5.0	25-40
10cm	3.5-5.5	100-150 (80-120)	5.0-8.0	90-150	2.0-3.0	100-150 (60-90)	3.0-5.0	80-90	5.0-15	80-150
			8.0-15*	50-150						

* If using a 10 cm dish for HEK293, the protocol to be used depends on whether number of cells are less than or greater than 8×10^6 (See Table 4 p.11).

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

◆ LRP (85 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 LRP before each use. Add 10 µl 2-ME per 1 ml of LRP. Dispense in a fume hood and wear appropriate protective clothing.

◆ SRP (40 ml)

Mix thoroughly before use.

◆ WRP (360 ml)

WRP is supplied as a concentrate.
Add 40 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.

◆ CRP (100 ml)

Use CRP for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to the Details of Protocol 8-3 (<3> p.20), 8-4 (<3> p.25).
Use immediately after preparation.

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

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

Table 3 Required volume of WRP and CRP

Number of Cartridges	WRP (QG-810/QG-800)	CRP (QG-810)	CRP (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 : WRP 8.0 ml, CRP 7.4 ml
QG-800 : WRP 8.0 ml, CRP 6.4 ml
Depending on the number of the Cartridges, add WRP and CRP.
Use WRP 2.25 ml and CRP 50 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 12.5 ml of WRP, 7.5 ml of CRP (QG-810) and 6.5 ml of CRP (QG-800) are required.
*Use appropriate tubes according to Table 1 (p.5).

8-2 Lysate Preparation Protocol

QuickGene RNA cultured cell HC kit S (RC-S2) corresponds to the extraction of total RNA from the number of cells corresponding to 6 to 10 cm dish.
Before performing an extraction, please select appropriate protocol referring to Table 4.

Table 4 Number of cells and corresponding protocols for various model cells

Dish Size	Cell example		Protocol	Referense page
	Cell species*1	Number of cells (×10^6)		
6 cm	HeLa	1.5-3.5	A	p.12
	HEK293	3.0-5.0		
	COS-7	0.5-1.5		
	NIH/3T3	1.0-2.5		
	HL60	3.0-5.0*3		
10 cm	HeLa	3.5-5.5	B	p.14
	HEK293	5.0-8.0*2		
	COS-7	2.0-3.0		
	NIH/3T3	3.0-5.0		
	HL60	5.0-15*3		
	HEK293	8.0-15*2	B'	p.16

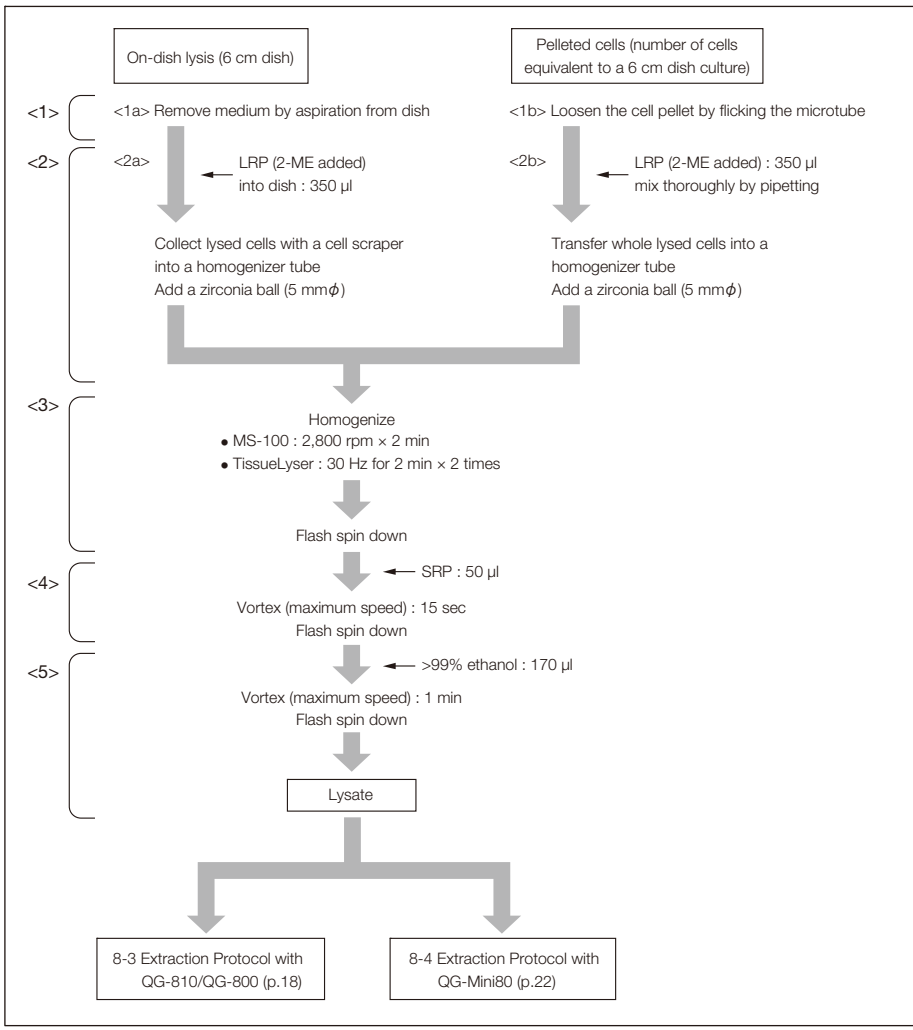
*1 : For extractions from cell types not included in Table 4, start the extraction using the number of cells equivalent to a sub-confluent layer on a 6 cm dish to examine the optimal number of cells.
*2 : If lysing a number of cells exceeding 8.0×10^6 directly on a dish, use Protocol B'. Pelleted cells can be processed up to 15×10^6 with Protocol B.
*3 : For non-adherent cells, the equivalent numbers of cells produced when cells are cultured in a volume of a culture medium corresponding to each size of dish are indicated.

[Important notes before starting]
- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow (p.12, 14, 16).
- 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 LRP 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]
- WRP is supplied as a concentrate. Check that 40 ml of >99% ethanol is added to WRP before starting an experiment.

Protocol A : Lysate Preparation Workflow

* For the number of cells corresponding to this protocol, see Table 4 (p.11).



Protocol A : Details of Lysate Preparation Workflow

This is a protocol for total RNA extraction from cells on a 6 cm dish or the corresponding number of pelleted cells.
Please refer to Table 4 (p.11) to check corresponding number of cells. We recommend that a cell count is performed before proceeding. If the number of cells is inappropriate, the extraction may end with the following results : clogging, low yield, and low purity. When clogging occurs, reduce the number of cells, and then try again.

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

<1b> 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, then loosen the cells by flicking the microtube.
- 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, then loosen the cells by flicking the microtube.

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.

<2> Add LRP to lyse cells.

Add 2-ME to LRP before use (p.9).

<2a> To lyse cells directly in the culture dish (On-dish lysis) :
Add 350 µl of LRP to the flask or dish, take off the cells with a cell scraper and mix it thoroughly. Transfer to an appropriate tube for each homogenizer. Put a 5 mmϕ zirconia ball in the tube in advance.

<2b> To lyse pelleted cells :
Add 350 µl of LRP. After pipetting several times, transfer the whole lysate into an appropriate tube for each homogenizer. Put a 5 mmϕ zirconia ball in the tube in advance.

<3> Homogenization can be performed by the following methods. Read the homogenizer manual carefully.

- TOMY Micro Smash MS-100 : Homogenize at 2,800 rpm for 2 min.
- QIAGEN TissueLyser : Homogenize twice at 30 Hz for 2 min.

Flash spin down for several seconds to remove drops from the inside of the lid.

Avoid the following situations that may cause damage to the homogenizer tube.

- Homogenizing at excessive speed.
- Not using the prescribed volume of LRP.
- Using balls other than a zirconia ball (5mmϕ) .

<4> Add 50 µl of SRP, and mix thoroughly by vortexing at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

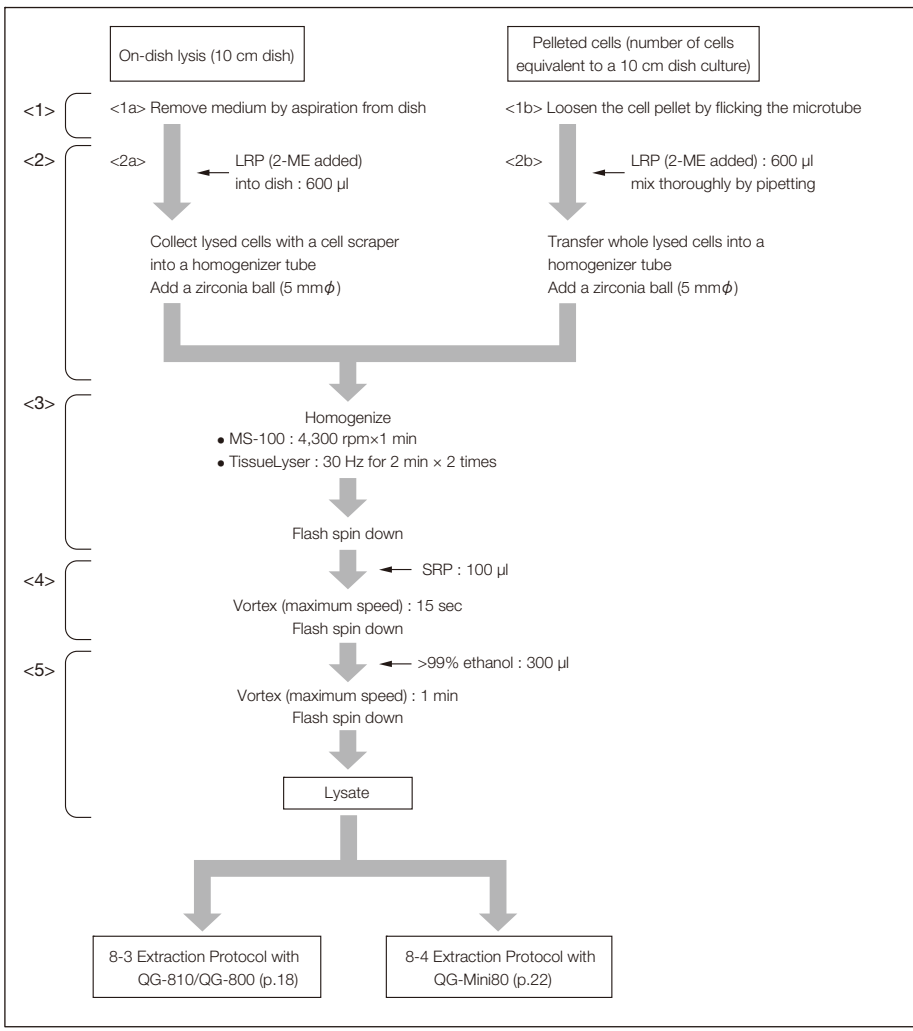
<5> Add 170 µl of >99% ethanol, and mix thoroughly by vortexing at the maximum speed for 1 min. Then flash spin down for several seconds to remove drops from the inside of the lid.

Perform the extraction operation quickly after completion of lysis.

QG-810/QG-800 (p.18)
QG-Mini80 (p.22)

Protocol B : Lysate Preparation Workflow

* For the number of cells corresponding to this protocol, see Table 4 (p.11).
* Use two Cartridges (CA) for each sample.



Protocol B : Details of Lysate Preparation Workflow

This is a protocol for total RNA extraction from cells on a 10 cm dish or the corresponding number of pelleted cells.
Please refer to Table 4 (p.11) to check corresponding number of cells. We recommend that a cell count is performed before proceeding. If the number of cells is inappropriate, the extraction may end with the following results : clogging, low yield, and low purity. When clogging occurs, reduce the number of cells, and then try again.

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

<1b> 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, then loosen the cells by flicking the microtube.
- 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, then loosen the cells by flicking the microtube.

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.

<2> Add LRP to lyse cells.

Add 2-ME to LRP before use (p.9).

<2a> To lyse cells directly in the culture dish (On-dish lysis) :
Add 600 µl of LRP to the flask or dish, take off the cells with a cell scraper and mix it thoroughly. Transfer to an appropriate tube for each homogenizer. Put a 5 mmϕ zirconia ball in the tube in advance.

<2b> To lyse pelleted cells :
Add 600 µl of LRP. After pipetting several times, transfer the whole lysate into an appropriate tube for each homogenizer. Put a 5 mmϕ zirconia ball in the tube in advance.

<3> Homogenization can be performed by the following methods. Read the homogenizer manual carefully.

- TOMY Micro Smash MS-100 : Homogenize at 4,300 rpm for 1 min.
- QIAGEN TissueLyser : Homogenize twice at 30 Hz for 2 min.

Flash spin down for several seconds to remove drops from the inside of the lid.

Avoid the following situations that may cause damage to the homogenizer tube.

- Homogenizing at excessive speed.
- Not using the prescribed volume of LRP.
- Using balls other than a zirconia ball (5mmϕ) .

<4> Add 100 µl of SRP, and mix thoroughly by vortexing at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

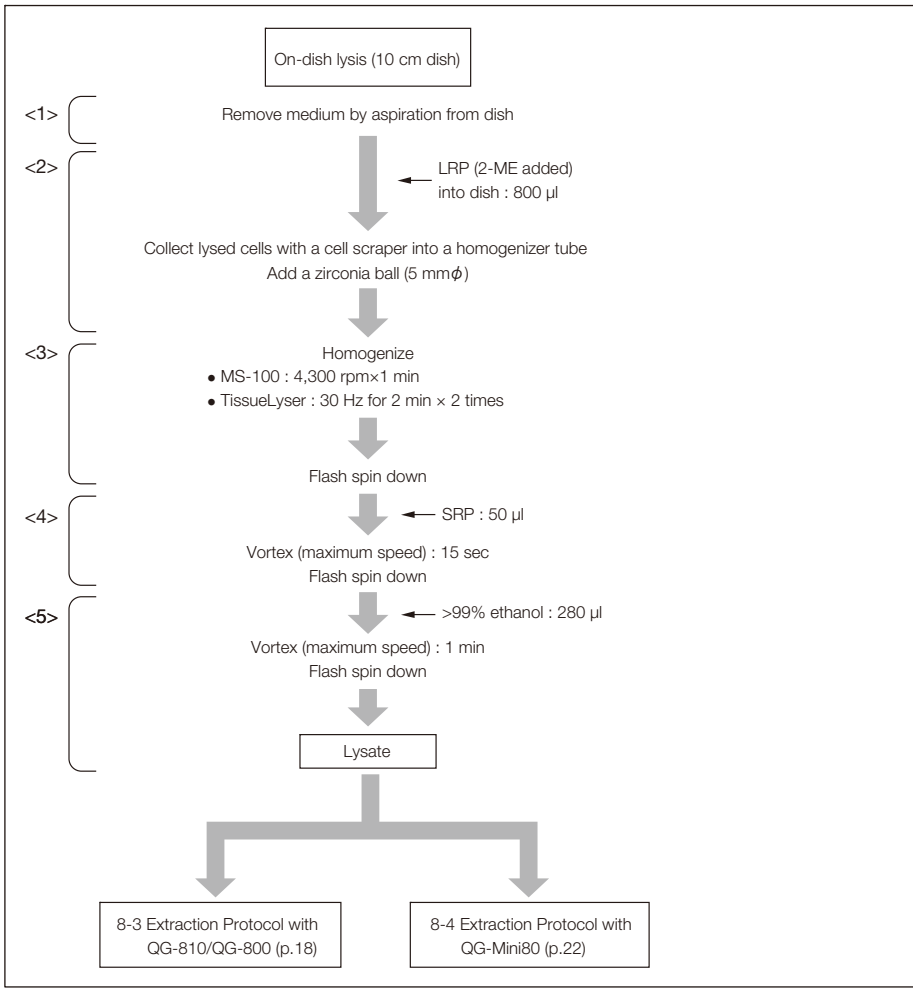
<5> Add 300 µl of >99% ethanol, and mix thoroughly by vortexing at the maximum speed for 1 min. Then flash spin down for several seconds to remove drops from the inside of the lid.

Perform the extraction operation quickly after completion of lysis.

QG-810/QG-800 (p.18)
QG-Mini80 (p.22)

Protocol B' : Lysate Preparation Workflow

* For the number of cells corresponding to this protocol, see Table 4 (p.11).
* Use two Cartridges (CA) for each sample.



Protocol B' : Details of Lysate Preparation Workflow

If clogging occurs with Protocol B, please use this protocol instead. This protocol is suitable for cell strains which produce high cell counts, for example, HEK293 (8×10^6 to 15×10^6) when cultured on a 10 cm dish, etc. Do not use Protocol B' for pelleted cells : use Protocol B even if the number of cells is in the range 8×10^6 to 15×10^6 .

<1> Remove the all of medium by aspirator.

<2> Add 800 µl of LRP to the flask or dish, take off the cells with a cell scraper and mix it thoroughly. Transfer to an appropriate tube for each homogenizer. Put a 5 mmϕ zirconia ball in the tube in advance.

Add 2-ME to LRP before use.

<3> Homogenization can be performed by the following methods. Read the homogenizer manual carefully.

- TOMY Micro Smash MS-100 : Homogenize at 4,300 rpm for 1 min.
- QIAGEN TissueLyser : Homogenize twice at 30 Hz for 2 min.

Flash spin down for several seconds to remove drops from the inside of the lid.

Avoid the following situations that may cause damage to the homogenizer tube.

- Homogenizing at excessive speed.
- Not using the prescribed volume of LRP.
- Using balls other than a zirconia ball (5mmϕ) .

<4> Add 50 µl of SRP, and mix thoroughly by vortexing at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

<5> Add 280 µl of >99% ethanol, and mix thoroughly by vortexing at the maximum speed for 1 min. Then 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.18)
QG-Mini80 (p.22)

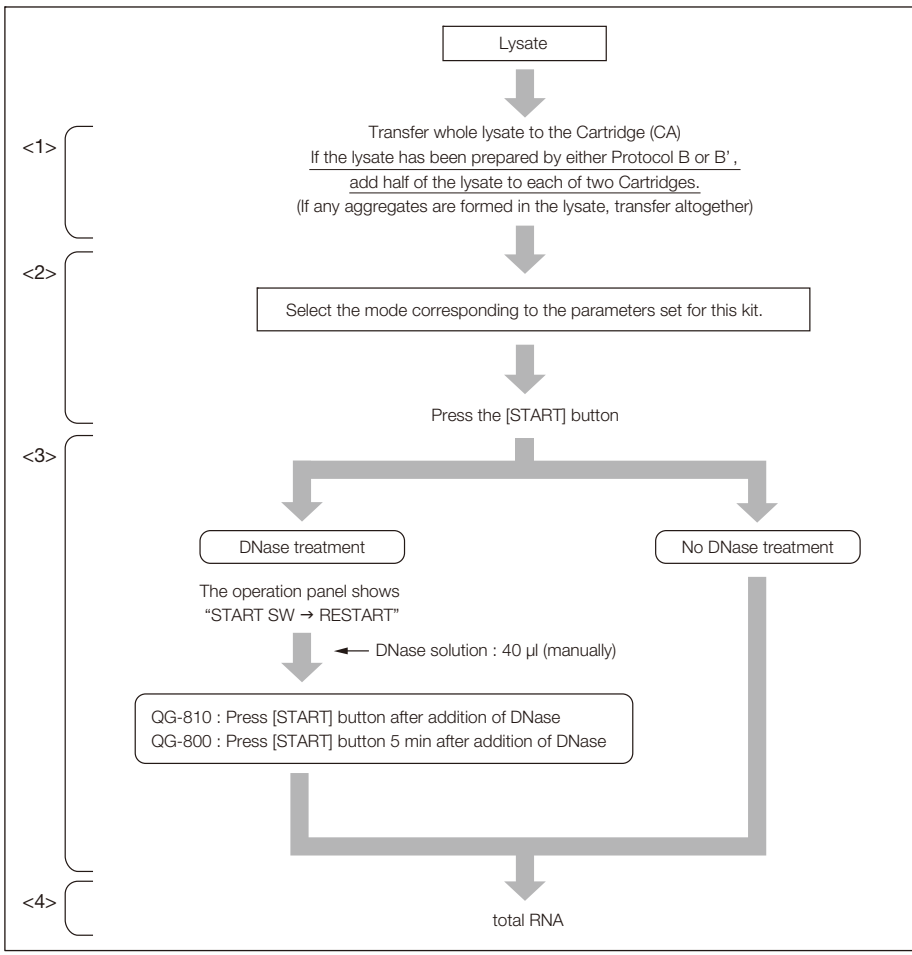
8-3 Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 40 ml of >99% ethanol is added to WRP before starting an experiment.
- Select the "RNA CELL PLUS" or "RNA CELL" mode as the extraction mode for QG-810 (see Appendix 1 p.35).
- Select the "RNA PLUS" or "RNA" mode as the extraction mode for QG-800 (see Appendix 2 p.37).
- All reagents, Cartridges (CA) and tubes are manufactured in clean rooms. Wear gloves during the experiments to avoid nuclease contamination.
- Refer to the User's Guide of QG-810/QG-800 for the details of setting Cartridges (CA), tubes and each reagent.
- Open the front cover of QG-810/QG-800 and set the Collection Tubes (CT) and the Waste Tubes (WT) in the Tube Holder (or Collection Tube Holder). Use the specified Cartridges (CA).
- Set WRP (>99% ethanol added) and CRP to QG-810/QG-800 referring to p.10.
- Incorrect Cartridge (CA) placement may result in the solution spilling or improper extraction.
- Press the [DISCHARGE] button after closed the front cover of QG-810/QG-800. Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- Any solution and waste fluid containing LRP 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

*Please change each parameter referring to the Appendix 1 and 2 (p.35, 37).
*When lysate is made with protocol B or B', two Cartridges (CA) of one sample are used.



Details of QG-810/QG-800 Workflow

<1> <Applying lysate> Set one Cartridge (CA) per sample (if the lysate has been prepared by Protocol A) or 2 Cartridges per sample (if the lysate has been prepared by Protocol B or B') on the QG-810/QG-800. Pipette the lysate several times, and then add the entire lysate to one Cartridge (Protocol A) or half the lysate to each of two Cartridges (Protocol B or B').

Pipetting needs to be done accurately. If the lysate was prepared using Protocol B or B', take care to ensure that precisely half of the lysate is applied to each of the two Cartridges. Inaccurate pipetting is likely to cause biased yields or result in clogging the filter.
Take care to avoid transferring the bubbles in the upper layer of the lysate to the Cartridge to avoid contamination of the sample. If aggregates have been formed in the lysate, the aggregates should be dispersed by pipetting, followed by transfer of the entire lysate (together with the floating 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.35), Appendix 2 (p.37). 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 5.

Table 5 Movement when you opened a front cover during extraction

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

*1 QG-810 : See User’s Guide of QG-810, “3.5 Operations to Restart Program from Pause” (p.28).

*2 QG-800 : The sample that was on the way of the extraction cannot be used again. Discard according to the applicable regulations. Refer to “Disposal of waste fluid and consumables when using potentially infectious samples of this handbook (p.6).

<3> <DNase treatment> If not using a DNase, proceed to step <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	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 DNase 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.35)

<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 6 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 (CA) will be 50 µl.
When using Protocol B or B’, the total RNA is collected into two Collection Tubes per sample. Combine the total RNA in the two tubes into one Collection Tube.

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 40 ml of >99% ethanol is added to WRP 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 (CA) to the Cartridge Holder. Confirm the Release Lever is positioned at the left-hand end of the Cartridge Holder.

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

- If after repeated the lysate or WRP remain in any Cartridges (CA), the Cartridges should be removed, and the steps shown in Troubleshooting ((4) p.30) taken.

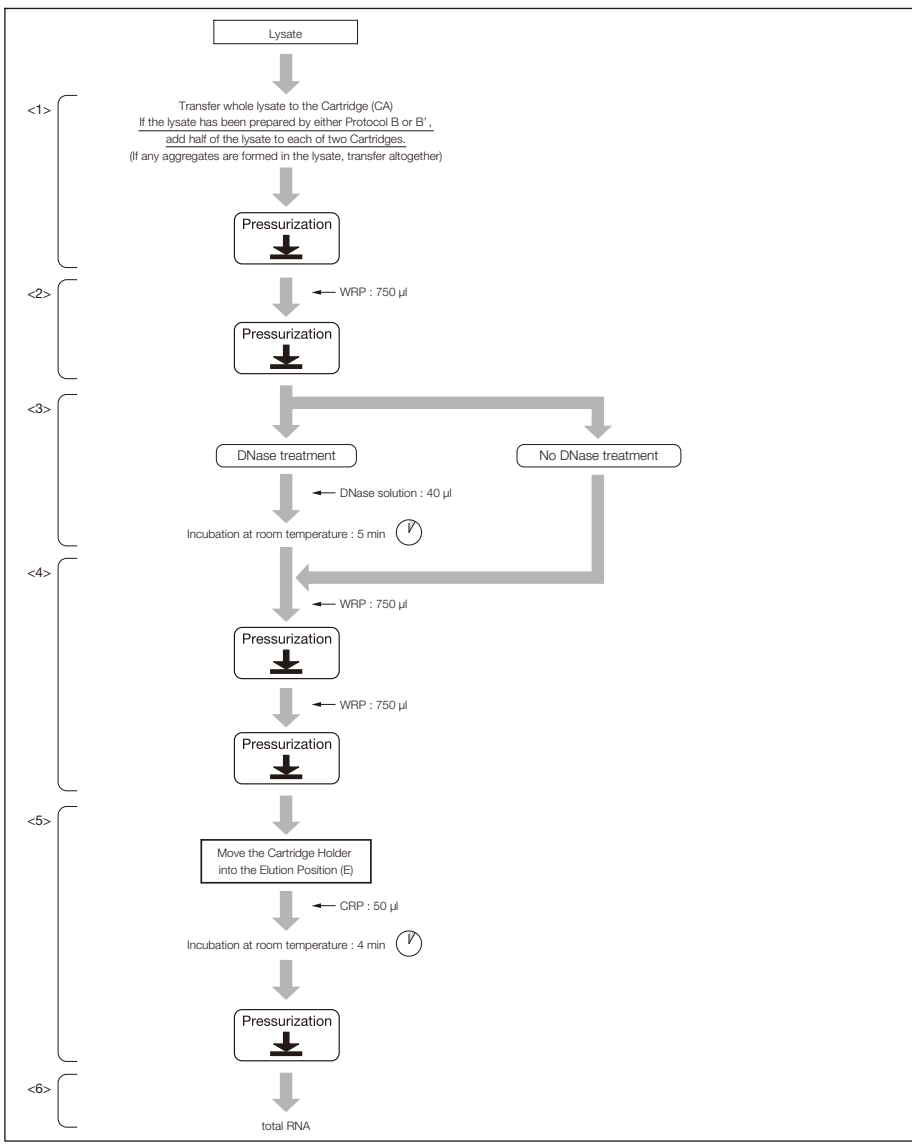
- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- Any solution and waste fluid containing LRP should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

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 (CA) and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and the Tube Holder from QG-Mini80.

*When lysate is made with protocol B or B’, two Cartridges (CA) of one sample are used.



Details of QG-Mini80 Workflow

<1> <Applying Lysate> Set one Cartridge (CA) per sample (if the lysate has been prepared by Protocol A) or 2 Cartridges per sample (if the lysate has been prepared by Protocol B or B') on the QG-Mini80. Pipette the lysate several times, and then add the entire lysate to one Cartridge (Protocol A) or half the lysate to each of two Cartridges (Protocol B or B'). 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.

Pipetting needs to be done accurately. If the lysate was prepared using Protocol B or B', take care to ensure that precisely half of the lysate is applied to each of the two Cartridges. Inaccurate pipetting is likely to cause biased yields or result in clogging the filter.
Take care to avoid transferring the bubbles in the upper layer of the lysate to the Cartridge to avoid contamination of the sample.
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 WRP to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WRP remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WRP 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>. 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	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 Cartridge Holder and the Tube Holder, add 40 µl of any DNases per Cartridge. After addition, set the Cartridge Holder and the Tube Holder to QG-Mini80, incubate at room temperature for 5 min.

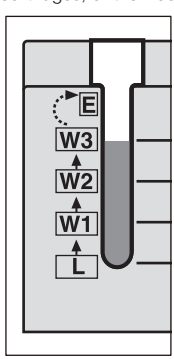
Avoid touching the filter in the Cartridge with the pipette tip during addition of DNase solution.
Pressurization should not be performed during incubation.
It is necessary to start pressurization after addition of WRP (>99% ethanol added) for the second wash (<4>).
It is necessary to start DNase treatment after the first wash.

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

Pressure application automatically stops in about 1 min. If any WRP 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 WRP to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WRP remains in the Cartridges and then return the Rotary Switch to the original position.

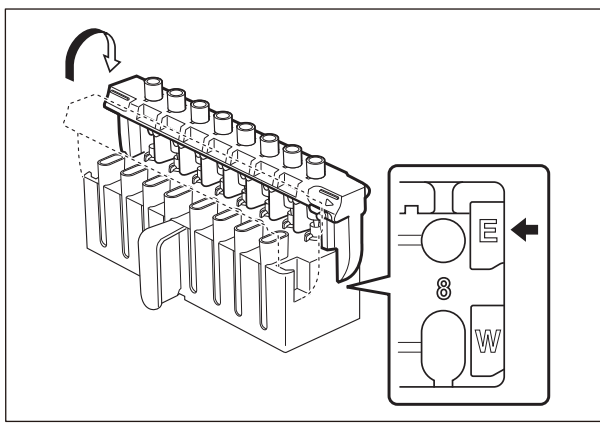
Pressure application automatically stops in about 1 min. If any WRP remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
After third wash, the waste fluid scale of the Tube Holder indicates [W3] position. (Refer to the following illustration)
Do not add WRP 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 (WT).



<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 CRP 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.

After incubating at room temperature for 4 min, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure that no CRP remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CRP 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 (CA). When sliding the Release Lever to the right-hand end, all Cartridges will fall down. Remove the Collection Tubes (CT) 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.

When using Protocol B or B', the total RNA is collected into two Collection Tubes per sample. Combine the total RNA in the two tubes into one Collection Tube.

If you do not use the eluted total RNA for any experiments immediately, after covering the Caps (CAP) on the Collection Tubes 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 HC kit S (RC-S2).

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

(1) Homogenization tube is ruptured :

Cause	Action
Used at over speed	Follow the designated speed.
Not enough solution volume for homogenization	Add the prescribed volume of LRP to cells, and add the whole volume of the mixture to a tube for homogenization.
Use of a ball other than that prescribed	Use one prescribed ball (zirconia, 5mm ϕ).
Use of a tube other than the prescribed microtubes for homogenization	Use the microtube appropriate for homogenizer.

(2) Low yield or no RNA obtained :

Cause	Action
Inadequate removal of culture medium from the dish	Any remaining culture medium decreases the concentration of LRP, leading to a decrease in the yield. Remove the culture medium from dish completely.
Inappropriate range of number of cells	Count the number of cells, and refer to Table 4 (p.11) to select an appropriate extraction protocol. If the number of cells is less than the application range, please try a QuickGene RNA cultured cell kit S (RC-S).
No addition of 2-ME to LRP	Dispense a required volume of LRP before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRP.
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, after cells are thawed, continue flicking the tube to completely loosen pellet.
Formation of a precipitate in LRP	Check before use that there is no precipitate formed in the LRP. If a precipitate is formed, dissolve fully by incubation at 37°C. Use after cooling back to room temperature.
Insufficient homogenization after addition of LRP	Check the speed and time of homogenizer as well as the addition of one zirconia ball to perform homogenization.
No addition of the prescribed volume of SRP or ethanol	Add the prescribed volume of SRP or >99% ethanol.
No addition of the prescribed volume of ethanol to WRP	Before using WRP for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.9.)
Incomplete addition of whole lysate to the Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Unequal dispensing of lysate to two Cartridges (CA)	Ensure any aggregates are evenly dispersed and then accurately dispense one-half of the lysate to each of two Cartridges. (Protcol B, B')
Excessive compression (**)	Compression should be discontinued immediately after the lysate or WRP has passed out of the Cartridge (CA) completely. To minimize variance in compression time, it is advisable to keep the amount of sample and other conditions as uniform as possible.

Cause	Action
Insufficient volume of CRP	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 CRP is 50 µl.
Inadequate volume of any buffer set (*)	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)	Make sure to add specified volume of DNase reaction buffer to DNase solution.
Perform pressurization without adding WRP to the Cartridge (CA) after incubation for 5 min following the addition of a DNase (**)	Add a DNase solution, incubate for 5 min, then add WRP to the Cartridge (CA) before pressurization.
Parameters (“ELUT DIP TM” (QG-810) or “CLCT DIP TM” (QG-800)) have not been changed to “240” (*)	Confirm that the parameters (“ELUT DIP TM” (QG-810) or “CLCT DIP TM” (QG-800)) have been changed to “240” referring to Appendix 1 and 2 (p.35, 37).
Not taking 4 min incubation time after addition of CRP (**)	Incubate for 4 min after addition of the CRP.
Use of too old WRP (*)	Check if WRP (>99% ethanol added) set in QG-810/QG-800 does not pass over 1 day.
RNA degradation	Refer to (5) “degradation”
Temperature of operation is high	Take all of operation at room temperature (15-28°C).
Rupturing of filter when adding a DNase (when using a DNase)	Add a DNase solution not to allow pipette tip to contact with a filter in the Cartridge (CA). In case of QG-810, take the Holder Carriage off, add DNase solution with confirming the end of tip from backside.
Clogged filter (Operation panel of QG-810 : –, QG-800 : ×)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA according to “Further Note” p.32.
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.

(3) Purity of RNA is low :

Cause	Action
No use of prescribed washing conditions	QG-810/QG-800 : Confirm the parameters have been changed, particularly that the setting of the parameter of “WASH VOL 1-5” and “WAS2 VOL 1-5” is correct (it should be “750”). QG-Mini80 : Wash the filter three times with 750 µl of WRP.
Foaming has occurred during addition of lysate to Cartridge (CA)	Bubbles formed during transfer of the lysate will persist and probably reduce the RNA purity. Take care to avoid the formation of bubbles when pipetting the lysate.
Use of reagents other than CRP to elute RNA	Use CRP to elute RNA.

(4) Clogging of Cartridge (CA) occurs :

Cause	Action
Use of too much amount of cells	Reduce the number of cells referring to Table 4 (p.11).
Inadequate resuspension of pelleted cell	Loosen pellet completely by flicking adequately. Particularly in the case of a frozen pellet, after cells are thawed, continue flicking the tube to completely loosen pellet.
Insufficient homogenization after addition of LRP	Check the speed and time of homogenizer as well as the addition of one zirconia ball before homogenization.
No addition of the prescribed volume of SRP or ethanol	Add the prescribed volume of SRP or >99% ethanol. If any sample is lost during homogenization , adjust volumes of SRP and >99% ethanol.
Insufficient homogenization after addition of ethanol	Vortex at the maximum speed after adding >99% ethanol.
Unequal dispensing of lysate to two Cartridges (CA)	Ensure any aggregates are evenly dispersed and then accurately dispense one-half of the lysate to each of two Cartridges. (Protcol B, B')
Insufficient pressurization (**)	Pressurize once more.
QG-810/QG-800 : Operation panel of "– (QG-810)" or "× (QG-800)" is displayed, and lysate or WRP is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRP completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA according to "Further Note" p.32.
No addition of the prescribed volume of ethanol to WRP	Before using WRP for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See 8-1 p.9).
Leaving Cartridges (CA) to stand after having discharged the fluid in it (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption.

(5) RNA degradation :

Cause	Action
No addition of 2-ME to LRP	Dispense required volume of LRP before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRP.
RNase contamination	Although all buffers, Cartridges (CA), 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 recommended RNase-free DNase. For details, inquire to each manufacturer.
Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

(6) 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 refer to the following (7) when the degradation of DNA is insufficient.
RNA degradation	Refer to (5) “RNA degradation”.
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.35-39). QG-Mini80 : Wash the filter three times with 750 µl of WRP.

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

Cause	Action
Use of unrecommended DNase	Use any one of recommended DNases (See [3]-1 p.5).
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	QG-810 : Confirm that the parameter “WAS2 WAIT T” is “5”. QG-800 or QG-Mini80 : Incubate at room temperature (15-28°C) for 5 min.
Required volume of DNase is not added	When preparing a DNase solution, check that a prescribed amount of DNase has been added.

(8) 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.

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

Cause	Action
Insufficient set of CRP or no operation of discharging (*)	Set the prescribed volume of CRP according to Table 3 (p.10). In addition, it is necessary to perform a discharging operation according to the User’s Guide of QG-810/QG-800.
No addition of CRP (**)	After insert the Cartridge Holder to the Elution Position (E), add 50 µl of CRP to Cartridge (CA).
No transfer of the Cartridge Holder to the Elution Position (E) when adding CRP (**)	When adding CRP, addition has to be started after the transfer of the Cartridge Holder to the Elution Position (E).

(10) 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.19) again.
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

b) If WRP remain in Cartridge (CA) :

Discard WRP remaining in the Cartridge (CA).
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) 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.24) 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 :

Discard WRP remaining in the Cartridge (CA).
For the recovery of RNA from a clogged filter in the Cartridge, proceed step 1) prescribed below.

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

1) Dispense the following volume of LRP (2-ME added) to a 1.5 ml microtube in advance. Select the recovery protocol corresponding to the protocol selected at the time of preparing the lysate.

   Protocol A 350 µl
   Protocol B 300 µl
   Protocol B' 400 µl

2) 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.
3) According to Figures 1 and 2, dismount filter from Cartridge (CA) with pushing rim of filter with the tip of tweezers.
4) Soak the dismounted filter into LRP (2-ME added) placed in 1.5 ml microtube, which has been prepared for use at 1), and incubate at room temperature for 10 min.
5) Vortex at the maximum speed for 1 min. Flash spin down for several seconds to remove the drops from inside of the lid.
6) Take out filter, and add the following volume of SRP to the residual solution according to the protocols.

   Protocol A 50 µl
   Protocol B 50 µl
   Protocol B' 25 µl

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

8) Add the following volume of >99% ethanol according to the protocols.

Protocol A	170 µl
Protocol B	150 µl
Protocol B'	140 µl

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

10) For all protocols, the entire solution prepared in step 9) should be added to one Cartridge (CA), followed by the extraction step <1> and subsequent steps described in p.19 or p.24.

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

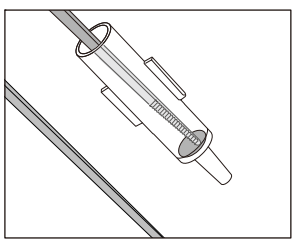
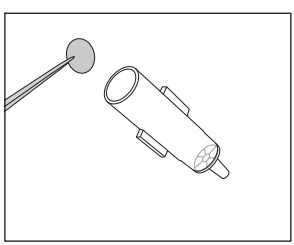


Figure 2 Appearance of a filter dismounted



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 PLUS” or “RNA CELL” is the following Table.
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 CELL PLUS (with DNase treatment)			RNA CELL (without DNase treatment)		
		PARAMETER	Check by user	Mode default	PARAMETER	Check by user	Mode default
1	BIND PEAK	120		120	120		120
2	WASH COUNT	1		1	3		3
3	WASH PEAK	110		110	110		110
4	WASH VOL1	750		500	750		500
5	WASH VOL2	750		500	750		500
6	WASH VOL3	750		500	750		500
7	WASH VOL4	750		500	750		500
8	WASH VOL5	750		500	750		500
9	WASH DIP TM	150		150	150		150
10	WAS2 WAIT T	5		5	0		0
11	WAS2 COUNT	2		2	0		0
12	WAS2 PEAK	110		110	110		110
13	WAS2 VOL1	750		500	750		500
14	WAS2 VOL2	750		500	750		500
15	WAS2 VOL3	750		500	750		500
16	WAS2 VOL4	750		500	750		500
17	WAS2 VOL5	750		500	750		500
18	ELUT VOL	50		100	50		100
19	ELUT PEAK	100		100	100		100
20	ELUT DIP TM	240		30	240		30

<How to change parameters>

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

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

   SETUP START
   ↓
   RNA CELL
   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 CRP 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 from “100” 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 CELL
   Returns to ready status.

Appendix 2 Setting of QG-800 Parameter

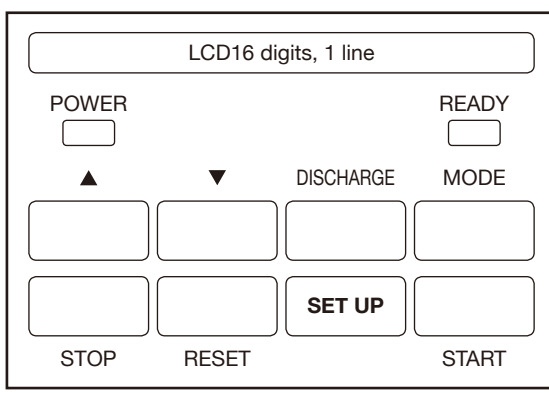
In the case of using a QG-800, the “RNA PLUS” and “RNA” modes are normally used for this kit. The “ISOLATE B” and “ISOLATE A” modes may also be used.
However, if using either the “ISOLATE B” or “ISOLATE A” modes, the operating parameters need to be changed as shown in the table below.
When changing the 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	“ISOLATE B” mode default	PARAMETER	Check by user	“RNA” mode default	“ISOLATE A” mode default
1	SAMP SPEED	10		10	10	10		10	10
2	SAMP PEAK	120		120	120	120		120	120
3	SAMP UP TIME	10		10	10	10		10	10
4	SAMP RETRY	160		160	160	160		160	160
5	SAMP LOWER	75		75	75	75		75	75
6	SAMP DOWN TM	25		25	25	25		25	25
7	SAMP R DN T	50		50	50	50		50	50
8	SAMP FALL	50		50	50	50		50	50
9	WASH COUNT	1		1	3	3		3	3
10	WASH SPEED	3		3	3	3		3	3
11	WASH PEAK	110		110	110	110		110	110
12	WASH UP TIME	10		10	10	10		10	10
13	WASH RETRY	140		140	140	140		140	140
14	WASH LOWER	70		70	70	70		70	70
15	WASH DOWN TM	15		15	15	15		15	15
16	WASH R DN T	50		50	50	50		50	50
17	WASH FALL	50		50	50	50		50	50
18	WASH VOL1	750		500	750	750		500	750
19	WASH VOL2	750		500	750	750		500	750
20	WASH VOL3	750		500	750	750		500	750
21	WASH VOL4	750		500	750	750		500	750
22	WASH VOL5	750		500	750	750		500	750
23	WASH DIP TM	150		150	0	150		150	0
24	WAS2 COUNT	2		2	0	0		0	0
25	WAS2 SPEED	3		3	3	3		3	3
26	WAS2 PEAK	110		110	110	110		110	110
27	WAS2 UP TIME	10		10	10	10		10	10
28	WAS2 RETRY	140		140	140	140		140	140
29	WAS2 LOWER	70		70	70	70		70	70
30	WAS2 DOWN TM	15		15	15	15		15	15
31	WAS2 R DN T	50		50	50	50		50	50
32	WAS2 FALL	50		50	50	50		50	50
33	WAS2 VOL1	750		500	750	750		500	750
34	WAS2 VOL2	750		500	750	750		500	750
35	WAS2 VOL3	750		500	750	750		500	750
36	WAS2 VOL4	750		500	750	750		500	750
37	WAS2 VOL5	750		500	750	750		500	750
38	CLCT VOL	50		100	200	50		100	200
39	CLCT COUNT	1		1	1	1		1	1
40	CLCT SPEED	5		5	5	5		5	5
41	CLCT PEAK	120		120	120	120		120	120
42	CLCT UP TIME	20		20	20	20		20	20
43	CLCT RETRY	160		160	140	160		160	140
44	CLCT LOWER	65		65	65	65		65	65
45	CLCT DOWN TM	15		15	15	15		15	15
46	CLCT R DN T	50		50	50	50		50	50
47	CLCT FALL	50		50	50	50		50	50
48	CLCT DIP TM	240		30	0	240		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.37).

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.

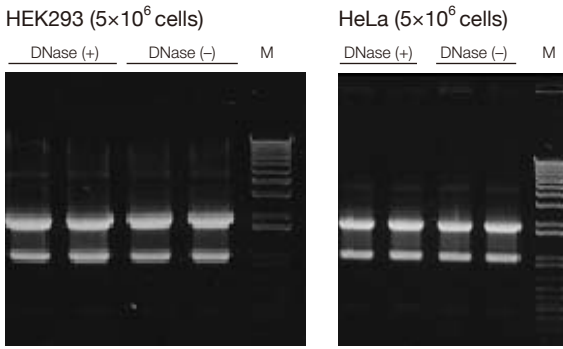
6. Return to “USER MODE”
   1) Turn power off.
   2) Take steps 1-1) through 1-4).
   3) Press [START] button to display “MENU : MAINTE MODE.” Then, press [▼] button to display “MENU : USER MODE.”
   4) While “MENU : USER MODE” is displayed, press [RESET] button and [SET UP] button (the unnamed button 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 Examples of the Data with QuickGene RNA cultured cell HC kit S (RC-S2)

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

Figure 3 illustrates the results of electrophoresis of total RNA extracted from various cultured cell lines with this kit.

Figure 3



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

● **RT-PCR**

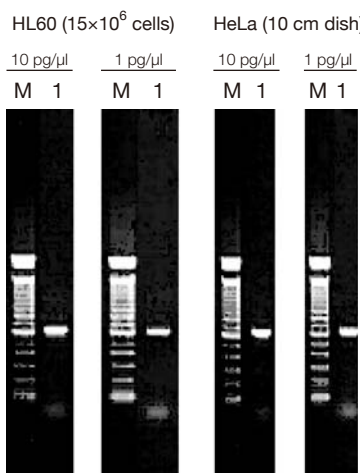
Figure 4 illustrates the results of RT-PCR amplification on the total RNA (when using a DNase) after extraction with this kit.

The extracted total RNA was diluted (10 pg/µl or 1 pg/µl). Then RT-PCR was performed with β-actin mRNA at following conditions.

<RT conditions>
Template : total RNA 500 ng
Enzyme : SuperScript Ⅱ (Invitrogen)

<PCR conditions>
Template : cDNA (equivalent to 10 pg/µl or 1 pg/µl total RNA)
Primer : β-actin primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

Figure 4



No.	Sample
1	total RNA sample extracted with this kit

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

RT-PCR amplification for β-actin was successfully performed using cDNA corresponding to 1 pg/µl total RNA.

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