KURABO

HANDBOOK

QuickGene RNA tissue kit S Ⅱ (RT-S2)

For extraction of total RNA from tissues

Ver.3.1

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 tissue samples. No hazardous organic solvents such as phenol and chloroform are used. RNA from 8 sets of tissue 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 10 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	LRT	85 ml
☐ Solubilization Buffer	SRT	40 ml
☐ Wash Buffer	WRT	120 ml
☐ Elution Buffer	CRT	100 ml
☐ Cartridges	CA	96
☐ Collection Tubes	CT	96
☐ Caps	CAP	96
☐ Waste Tubes	WT	96

2-2 Storage Conditions

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

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

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

*Prepare if necessary

- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega : Cat. No. M6101)
  - 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 *1 (Large/Small sets)
- Micropipettes and tips (RNase-free)
- 1.5 ml microtubes (RNase-free)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Homogenizer
  a. Ball mill homogenizer (TOMY Micro Smash MS-100 / QIAGEN TissueLyser)
  b. Rotor-Stator homogenizer (KINEMATICA AG Polytron PT3100, etc.)
  c. Pestle homogenizer for microtube*2
  (KIMBLE KONTES PELLET PESTLE with tube 1.5 ml (Cat.No. 749520-0090) /
  PELLET PESTLE Cordless Motor (Cat.No.749540-0000), etc.)
- Tubes appropriate for homogenizer
  a. Ball mill homogenizer
  TOMY Micro Smash MS-100 : TOMY 2ml tube (Cat. No.TM-625)*3
  QIAGEN TissueLyser : TreffLab 2.0ml click cap (Cat. No.96.9329.9.01)
  b. Rotor-Stator homogenizer
  2ml tubes, etc.
  c. Pestle homogenizer for microtube
  1.5ml tubes (attached with Pestle), etc.
- Ball (zirconia 5mmϕ) (For ball mill homogenizer only)
- Microcentrifuge (c.a. 17,000 × g (15,000 rpm))

*1 Centrifuge tubes are used with the QG-810/QG-800 as containers for WRT (>99% ethanol added) and CRT. They are unnecessary when QG-Mini80 is used.
*2 Using motor is recommended for Pestle homogenization.
*3 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 WRT)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CRT)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WRT)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CRT)	BD Falcon™ 50 ml conical tube

4. Safety Warnings

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

◆ LRT (Lysis Buffer)
- **Harmful if ingested.**
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ SRT (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.

◆ WRT (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.

◆ CRT (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 LRT at high temperature should be avoided.

◆ Any solution and waste fluid containing LRT 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

- QuickGene RNA tissue kit S Ⅱ (RT-S2) corresponds to total RNA extraction from 5 to 30 mg of mammalian tissue sample.

Table 2 Maximum amounts of normal mouse tissue for each homogenizer
Balb/c mouse (female, 7-week old)

Tissue	Ball mill	Rotor-Stator	Pestle
Liver	30 mg	15 mg	15 mg
Brain	40 mg	40 mg	20 mg
Lung	30 mg	15 mg	15 mg
Kidney	30 mg	5 mg	×
Spleen	30 mg	20 mg	10 mg
Thymus	30 mg*	5 mg	5 mg
Heart	30 mg*	5 mg	×

× : out of application

* Since Heart and Thymus are more difficult to homogenize, stronger condition must be applied. For example, in the case of TOMY Micro Smash MS-100, prolonging homogenization time is strongly recommended (see Table 5 p.15). In case of insufficient homogenization, clogging Cartridge may occur.

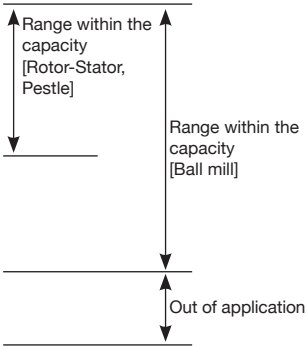
- If you use QuickGene RNA tissue kit S Ⅱ (RT-S2) for the first time, start with 10 mg of tissue. Performing a preliminary test is recommended.
- Before tissue samples are homogenized, check the maximum amounts of sample that can be processed with each homogenizer in Table 2.
- Do not overload the Cartridge (CA), as this will significantly reduce RNA yield and quality. In the worst case, the Cartridge may clog.
- The maximum amount may vary depending on the sample species, condition, type and homogenization condition.
- Fresh or frozen tissue (–80°C) can be used. In case the tissue is not immediately used, the tissue should be flash frozen with liquid nitrogen and stored at –80°C.
- In the case of using a frozen tissue sample, weigh quickly before thawing.
- Do not allow any tissue to stand at room temperature, or do not use any tissue once thawed.

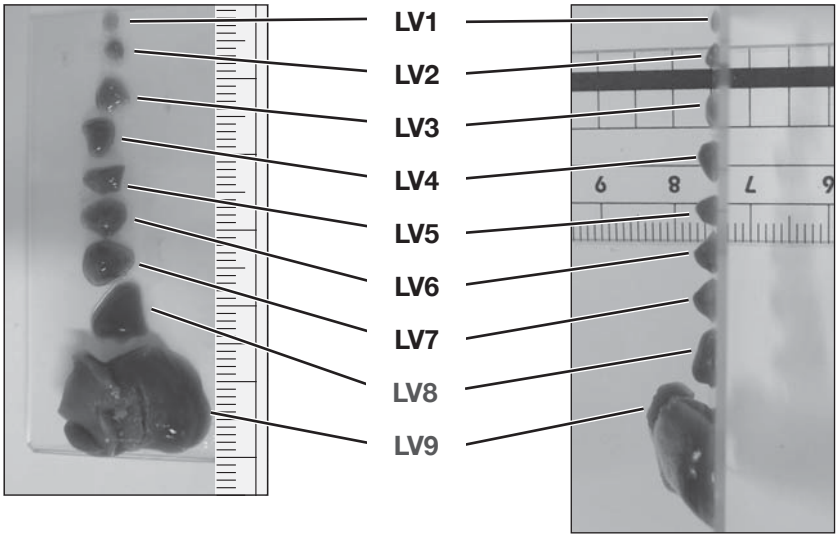
- Figure 1 shows the relationship between the weight and the dimensions of samples of normal mouse tissue (liver and lung). Check the sample tissue weight and select an extraction protocol corresponding to the sample weight.
(15 mg to 30 mg : p.14, 5 to 15 mg : p.17)

Figure 1 : Relationship between the weight and the dimensions of samples of normal mouse tissue (liver and lung)
Examples for normal tissues of Balb/c mouse (female, 7-week old)

Liver

No.	Weight	Long axis	Short axis	Height
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm





*LV2 to LV7 correspond to the 5 to 30 mg size. LV9 corresponds to the whole liver size of mouse.

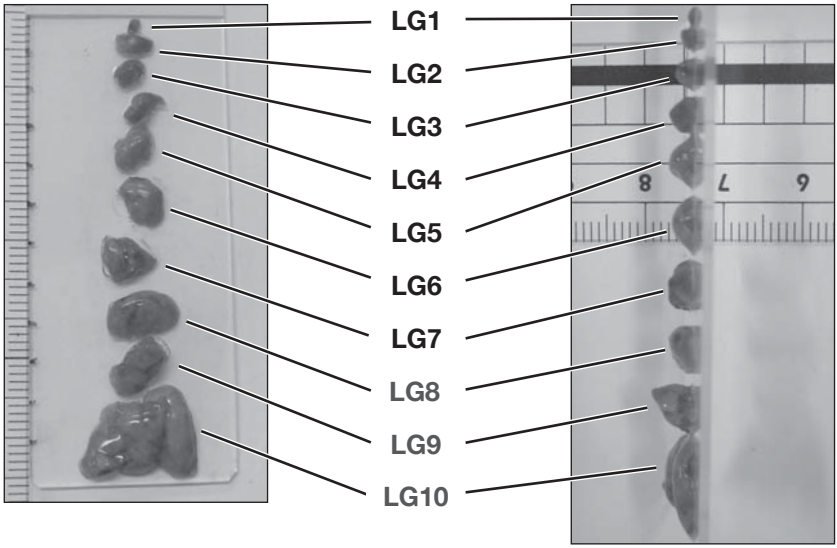
Lung

No.	Weight	Long axis	Short axis	Height
LG1	1.7 mg	1.5 mm	1.5 mm	1.0 mm
LG2	6.8 mg	5.0 mm	2.5 mm	2.0 mm
LG3	8.7 mg	4.5 mm	3.0 mm	2.5 mm
LG4	15.3 mg	5.0 mm	2.5 mm	2.5 mm
LG5	20.8 mg	6.0 mm	4.0 mm	2.5 mm
LG6	25.2 mg	7.0 mm	5.0 mm	2.5 mm
LG7	30.2 mg	7.0 mm	5.5 mm	3.0 mm
LG8	40.4 mg	9.5 mm	6.0 mm	3.0 mm
LG9	46.2 mg	8.0 mm	5.0 mm	4.0 mm
LG10	134.3 mg	15.0 mm	11.0 mm	4.0 mm

Range within the capacity [Rotor-Stator, Pestle]

Range within the capacity [Ball mill]

Out of application



*LG2 to LG7 correspond to the 5 to 30mg size. LG10 corresponds to the whole lung size of mouse.

◆ Use of Reagent

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

◆ Procedure of Extraction

- Use QuickGene RNA tissue kit S Ⅱ (RT-S2) at room temperature (15-28°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- This kit is designed for extractions using 100 µl of CRT. The volume of CRT may be changed, however, the efficiency of elution may also change.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : 8-3 (p.19), Appendix 1 (p.36), Appendix 2 (p.38)
  QG-Mini80 : 8-4 (p.23)
- Refer to QuickGene User's Guide for the details.

<Prevention against RNase contamination>

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

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene RNA tissue kit SⅡ (RT-S2) is evaluated routinely on a lot-to-lot uniformity.
- QuickGene RNA tissue kit SⅡ (RT-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

Table 3 shows the example of yields and purifies of total RNA obtained with this kit.

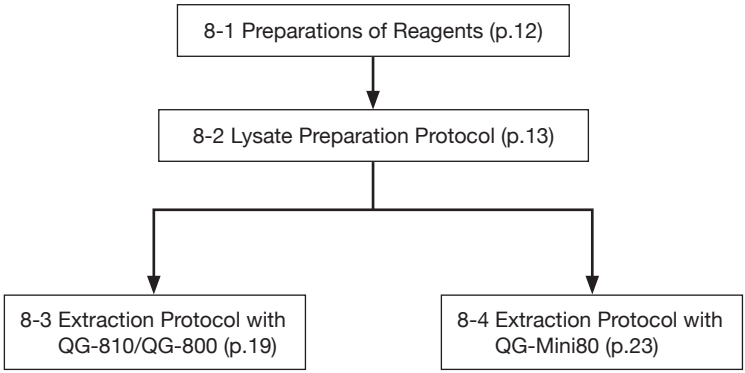
Table 3 Examples of yields and purities of total RNA obtained from normal tissues of Balb/c□ mouse (female, 7-week old)
(homogenized with a TOMY Micro Smash MS-100; with DNase treatment)

Tissue	Tissue amount	Yield of total RNA recovered	A260/280
Liver	30 mg	100-120 µg	2.2
Brain	30 mg	15-20 µg	2.1
Lung	30 mg	20-25 µg	2.2
Kidney	30 mg	50-60 µg	2.3
Spleen	30 mg	40-50 µg	2.2
Thymus	30 mg	40-60 µg	2.2
Heart	30 mg	15-20 µg	2.2

- Yields and purities may vary depending on the sample species, condition and tissue type.

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

◆ LRT (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 LRT before each use. Use 500µl of LRT per 1 Cartridge (CA). Add 10 µl 2-ME per 1 ml of LRT. Dispense in a fume hood and wear appropriate protective clothing.

◆ SRT (40 ml)

Mix thoroughly before use.

If the precipitates are formed during storage dissolve them fully by incubation at 37°C.

Cool down it to room temperature before use.

◆ WRT (120 ml)

WRT is supplied as a concentrate.

Add 280 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.

◆ CRT (100 ml)

Use CRT for elution of RNA.

◆ DNase solutions (when using a DNase treatment)

Prepare according to the Details of Workflow (<3> p.21 or <3> p.26).

Use immediately after preparation.

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

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

Table 4 Required volume of WRT and CRT

Number of Cartridges	WRT (QG-810/QG-800)	CRT (QG-810)	CRT (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 : WRT 8.0 ml, CRT 7.4 ml

QG-800 : WRT 8.0 ml, CRT 6.4 ml

Depending on the number of the Cartridges, add WRT and CRT.

Use WRT 2.25 ml and CRT 100 µl per 1 Cartridge.

For example, in case of using 2 Cartridges, 12.5 ml of WRT, 7.6 ml of CRT (QG-810) and 6.6 ml of CRT (QG-800) are required.

*Use appropriate tubes according to Table 1 (p.5).

8-2 Lysate Preparation Protocol

QuickGene RNA tissue kit S Ⅱ (RT-S2) corresponds to the extraction of total RNA from 5 to 30 mg of animal tissues.

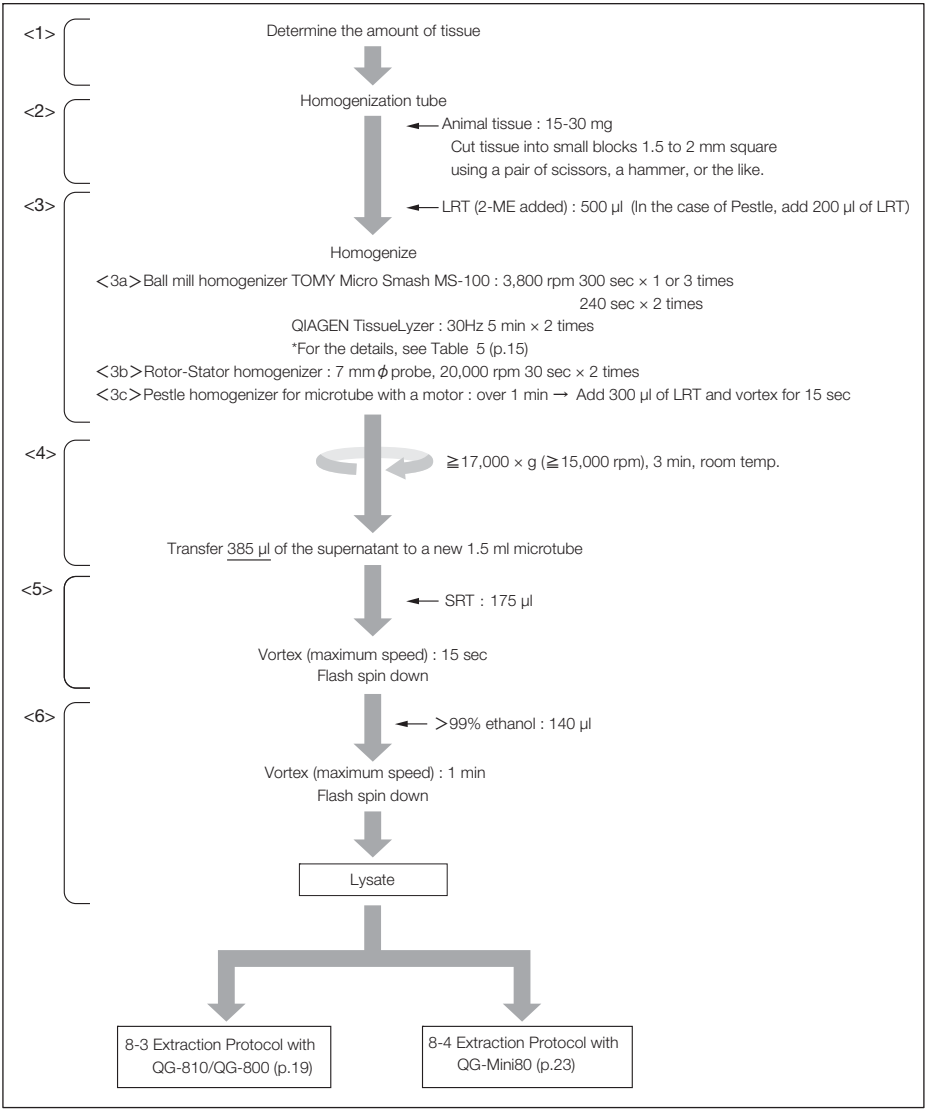
[Important notes before starting]
- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow (p.14, 17).
- To prevent cross-contamination, it is recommended to change the pipette tips between all liquid transfers.
- All steps of the protocol should be performed at room temperature (15-28°C).
- During the procedure, work quickly without interruption.
- Any solution and waste fluid containing LRT 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]
- WRT is supplied as a concentrate. Check that 280 ml of >99% ethanol is added to WRT before starting an experiment.

Choose the appropriate protocol depending on the amount of tissue. Each protocol is optimized for each amount.

15-30 mg : p.14
5-15 mg : p.17

For 15-30 mg tissue : Lysate Preparation Workflow



Details of Lysate Preparation Protocol : For 15-30 mg

<1> Prepare a fresh or frozen tissue sample excised from animal.
Determine the amount of a tissue.

Excessive amounts of tissue may result in clogging, low yield, and low purity. In the case of clogging, reduce amount of the sample. Refer to Table 2 (p.7).
If your material is not listed in Table 2 (p.7), we recommend starting with no more than 10 mg and use the protocol for 5-15 mg (p.17).

<2> After cutting the tissue in pieces (1.5-2 mm cubes) with scissors or a hammer etc., weigh the tissues, and transfer into a tube appropriate for each homogenizer (see 3-[2] p.4). If using a ball mill homogenizer, put a zirconia ball (5mmϕ) into the tube before transferring the tissue.

Weighing the tissue should be done rapidly to avoid RNA degradation. Keep the tissue frozen until adding LRT (2-ME added). We recommend setting the tubes in dry ice or liquid nitrogen before adding LRT.

<3> Add 500 µl of LRT (2-ME added), and then homogenize the tissue. When a pestle is used, 200 µl of LRT is added first, followed by the addition of 300 µl (see step <3c>).

Methods <3a> through <3c>, shown below, are available for crushing and homogenizing the tissue. The maximum amount of tissue that can be processed varies depending on the method. Refer to Table 2 (p.7) before starting the experiment. The operating instructions for each homogenizer should be read carefully before using the device for homogenization.
Add LRT into the frozen tube carefully, as the LRT might not splash out or cracks the tube if added rapidly.

<3a> Ball mill homogenizer

Put the weighed tissue sample into the appropriate tube (3-[2] p.4). Put a zirconia ball (5mmϕ) in advance. Add 500 µl of LRT, and homogenize thoroughly.
Speed and processing time may vary depending on conditions of tissue and kind of tissue. Table 5 shows some examples of homogenization conditions. It is necessary to use the appropriate tube (see section 3 p.4)

Table 5 Suggested homogenization conditions suitable for use with 15 to 30 mg of tissue

	TOMY Micro Smash MS-100	QIAGEN TissueLyzer
Liver / Brain / Lung / Kidney / Spleen	3,800 rpm 300 sec	30 Hz 5 min × 2 times
Thymus*	3,800 rpm 240 sec × 2 times*	
Heart	3,800 rpm 300 sec × 3 times	

*In the case of Thymus, TOMY Micro Smash MS-100R (with a cooler) may yield more compared with MS-100.

Avoid the following situations that may cause damage to the homogenizer tube.
- Homogenizing at excessive speed.
- Not using the prescribed volume of LRT.
- Using balls other than a zirconia ball (5mmϕ).

<3b> Rotor-Stator homogenizer

Lower amount of tissue can be processed compared with a Ball mill homogenizer (See Table 2 p.7).

Put the weighed tissue sample into the appropriate microtube. Use 2 ml microtube for 7 mmϕ probe. Add 500 µl of LRT and homogenize rapidly at 20,000 rpm, for 30 sec, 2 times.

In the case of 10 mmϕ probe or above, use appropriate size of tube. Homogenization speed and processing time may need to adjust for each sample type of tissue. If tissue debris is floating in the supernatant, increase the processing time further. Careful the bubble does not overflow from the tube. We recommend homogenize to be done applying the probe to the wall of the tube. Sometimes, it becomes difficult to foam.

<3c> Pestle homogenizer for microtube

Some type of tissue cannot homogenize completely with Pestle (See Table 2 p.7).

Put the weighed tissue sample into 1.5 ml microtube, add 200 µl of LRT and homogenize them over 1 min by Pestle.

To homogenize sample completely, press-down the Pestle to the bottom of the tube several times.

Add 300 µl of LRT after the homogenization, and mix thoroughly by vortexing for 15 sec.

Insufficient homogenization may result a clogging even though amount of tissue is below the amount of Table 2 (p.7).

<4> Centrifuge at ≧17,000 × g (≧15,000 rpm) at room temperature for 3 min to remove tissue debris. Transfer 385 µl of supernatant to new 1.5 ml microtube.

Do not take any debris from the bottom of tube when transferring the supernatant.
If tissue debris is floating in the supernatant after centrifugation, increase the speed and centrifugation time further.
If you have to interrupt the experiment, an interruption of 1 hour before step <5> would be allowable without any influence on the yield of total RNA. After step <5>, proceed without interruption.

<5> Add 175 µl of SRT 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.

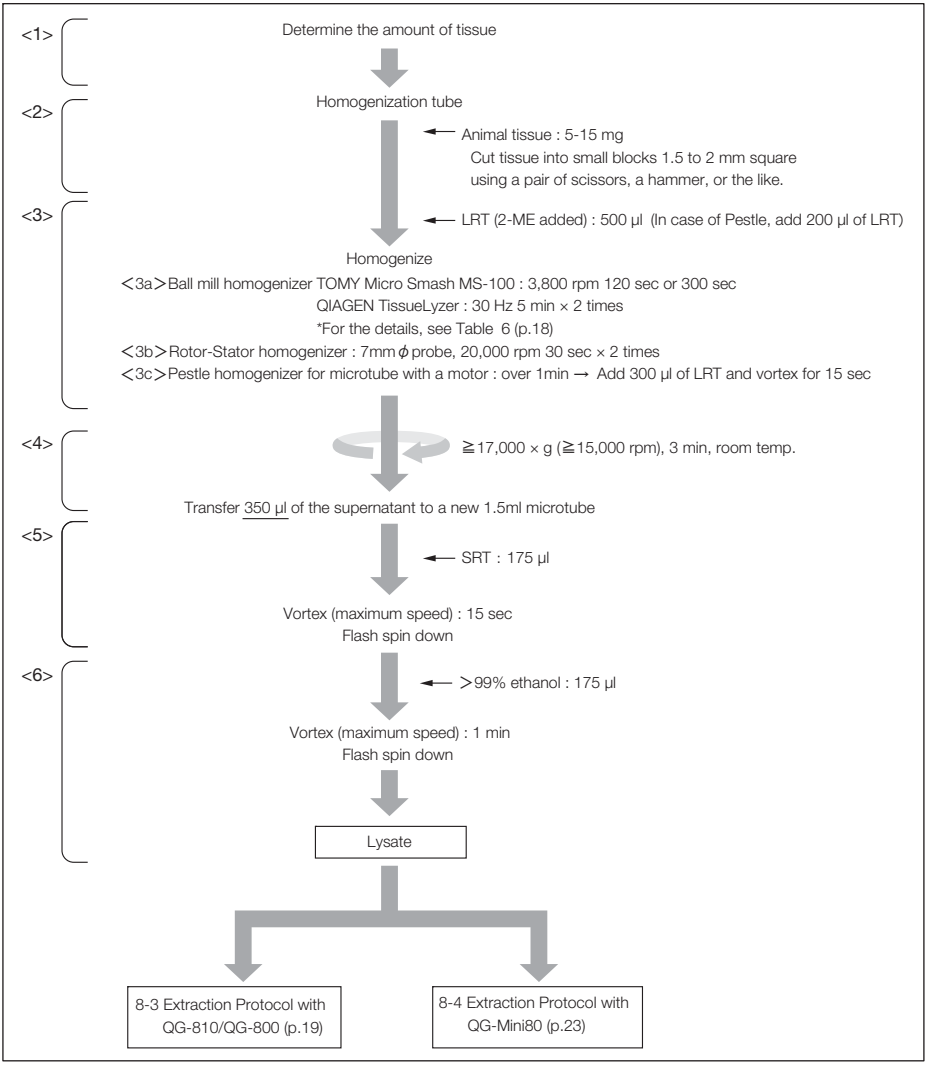
If any sample is lost during homogenization at steps <3> & <4>, adjust the volumes of SRT and >99% ethanol (step <6>) ; the volume ratio “homogenate : SRT : ethanol” should be kept to “11 : 5 : 4”.

<6> Add 140µl of >99% ethanol and thoroughly by vortexing at the maximum speed for 1min. Flash spin down for several seconds to remove drops from the inside of the lid.

Insufficient vortexing may cause low yield.

Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.19)
QG-Mini80 (p.23)

For 5-15 mg tissue : Lysate Preparation Workflow



Details of Lysate Preparation Protocol : For 5-15 mg

<1> Prepare a fresh or frozen tissue sample excised from animal.
Determine the amount of a tissue.

Excessive amounts of tissue may result in clogging, low yield, and low purity. In the case of clogging, reduce amount of the sample. Refer to Table 2 (p.7).
If your material is not listed in Table 2 (p.7), we recommend starting with no more than 10 mg.

<2> After cutting the tissue in pieces (1.5-2 mm cubes) with scissors or a hammer etc., weigh the tissues, and transfer into a tube appropriate for each homogenizer (see 3-[2] p.4). If using a ball mill homogenizer, put a zirconia ball (5mmϕ) into the tube before transferring the tissue.

Weighing the tissue should be done rapidly to avoid RNA degradation. Keep the tissue frozen until adding LRT (2-ME added). We recommend setting the tubes in dry ice or liquid nitrogen before adding LRT.

<3> Add 500 µl of LRT (2-ME added), and then homogenize the tissue. When a pestle is used, 200 µl of LRT is added first, followed by the addition of 300 µl (see step <3c>).

Methods <3a> through <3c>, shown below, are available for crushing and homogenizing the tissue. The maximum amount of tissue that can be processed varies depending on the method. Refer to Table 2 (p.7) before starting the experiment. The operating instructions for each homogenizer should be read carefully before using the device for homogenization.
Add LRT into the frozen tube carefully, as the LRT might not splash out or cracks the tube if added rapidly.

<3a> Ball mill homogenizer

Put the weighed tissue sample into the appropriate tube (3-[2] p.4). Put a zirconia ball (5mmϕ) in advance. Add 500 µl of LRT, and homogenize thoroughly.

Speed and processing time may vary depending on conditions of tissue and kind of tissue. Table 6 shows some examples of homogenization conditions. It is necessary to use the appropriate tube (see section 3 p.4)

Table 6 Suggested homogenization conditions suitable for use with 5 to 15 mg of tissue

	TOMY Micro Smash MS-100	QIAGEN TissueLyzer
Liver / Brain / Lung	3,800 rpm 120 sec	30 Hz 5 min × 2 times
Kidney / Spleen / Thymus / Heart	3,800 rpm 300 sec	

Avoid the following situations that may cause damage to the homogenizer tube.
- Homogenizing at excessive speed.
- Not using the prescribed volume of LRT.
- Using balls other than a zirconia ball (5mmϕ).

<3b> Rotor-Stator homogenizer

Put the weighed tissue sample into the appropriate tube. Use 2 ml microtube for 7 mmϕ probe. Add 500 µl of LRT and homogenize rapidly at 20,000 rpm, for 30 sec, 2 times.

In the case of 10 mmϕ probe or above, use appropriate size of tube. Homogenization speed and processing time may need to adjust for each sample type of tissue. Careful the bubble does not overflow from the tube. We recommend homogenize to be done applying the probe to the wall of the tube. Sometimes, it becomes difficult to foam.

<3c> Pestle homogenizer for microtube

Some type of tissue cannot homogenize completely with Pestle (See Table 2 p.7).
Put the weighed tissue sample into 1.5 ml microtube, add 200 µl of LRT and homogenize them over 1 min by Pestle.
To homogenize sample completely, press-down the Pestle to the bottom of the tube several times.
Add 300 µl of LRT after the homogenization, and mix thoroughly by vortexing for 15 sec.

Insufficient homogenization may result a clogging even though amount of tissue is below the amount of Table 2 (p.7).

<4> Centrifuge at ≧17,000 × g (≧15,000 rpm) for 3 min at room temperature to remove tissue debris. Transfer 350 µl of supernatant to new 1.5 ml microtube.

Do not take any debris from the bottom of tube when transferring the supernatant.
If tissue debris is floating in the supernatant after centrifugation, increase the speed and centrifugation time further.
If you have to interrupt the experiment, an interruption of 1 hour before step <5> would be allowable without any influence on the yield of total RNA. After step <5>, proceed without interruption.

<5> Add 175 µl of SRT 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.

If any sample is lost during homogenization at steps <3> & <4>, adjust the volumes of SRT and >99% ethanol (step <6>) ; the volume ratio "homogenate : SRT : ethanol" should be kept to "2 : 1 : 1".

<6> Add 175 µl of >99% ethanol and thoroughly by vortexing at the maximum speed for 1min. Flash spin down for several seconds to remove drops from the inside of the lid.

Insufficient vortexing may cause low yield.

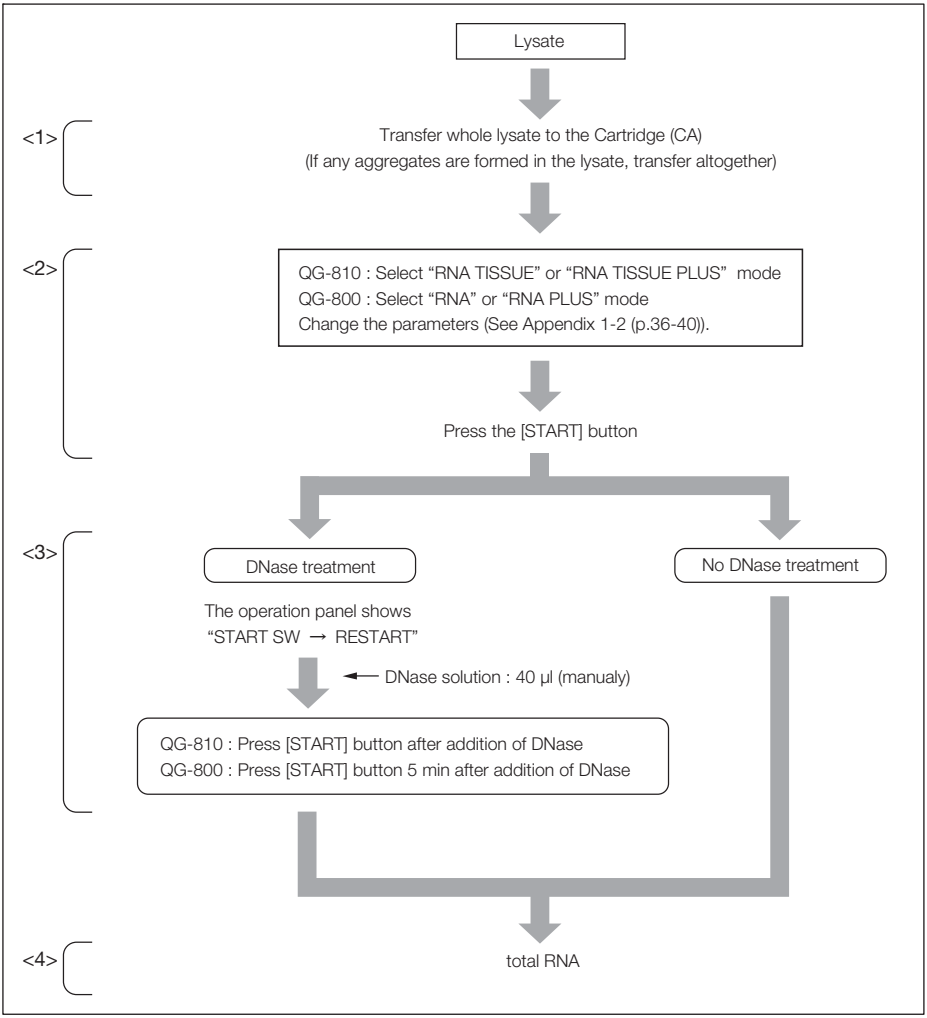
Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.19)
QG-Mini80 (p.23)

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 280 ml of >99% ethanol is added to WRT before starting an experiment.
- Select "RNA TISSUE", "RNA TISSUE PLUS" mode as the extraction mode for QG-810. (see Appendix 1 p.36)
- Select "RNA", "RNA PLUS" mode as the extraction mode for QG-800. (see Appendix 2 p.38)
- 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 WRT (>99% ethanol added) and CRT to QG-810/QG-800 referring to p.12.
- 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 LRT should not be mixed with bleach.
- When using potentially infectious samples for experiments, dispose them according to the applicable regulations.

QG-810/QG-800 Workflow



Details of QG-810/QG-800 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate (See section 8-2 p.13) 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.36), Appendix 2 (p.38). 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 7.

Table 7 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 Operrations 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	Manufacturer	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Life Technologies	AM2222	2	40 U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

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

Preparation 1)

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

Preparation 2)

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

Preparation 3)

2.7 Kunitz units/µl DNase *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.36)

<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 8 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 100 µl.
The volume of CRT can be reduced to 50 µl. At that time, it is recommended that the “ELUT DIP TM” parameter be reset to “240”.
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 280 ml of >99% ethanol is added to WRT 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 the 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 WRT (>99% ethanol added), confirm that the Wash Label on the Tray can be entirely seen.
- When pressuring CRT, confirm that the Wash Label on the Tray can not be seen, being hidden below the Tube Holder.

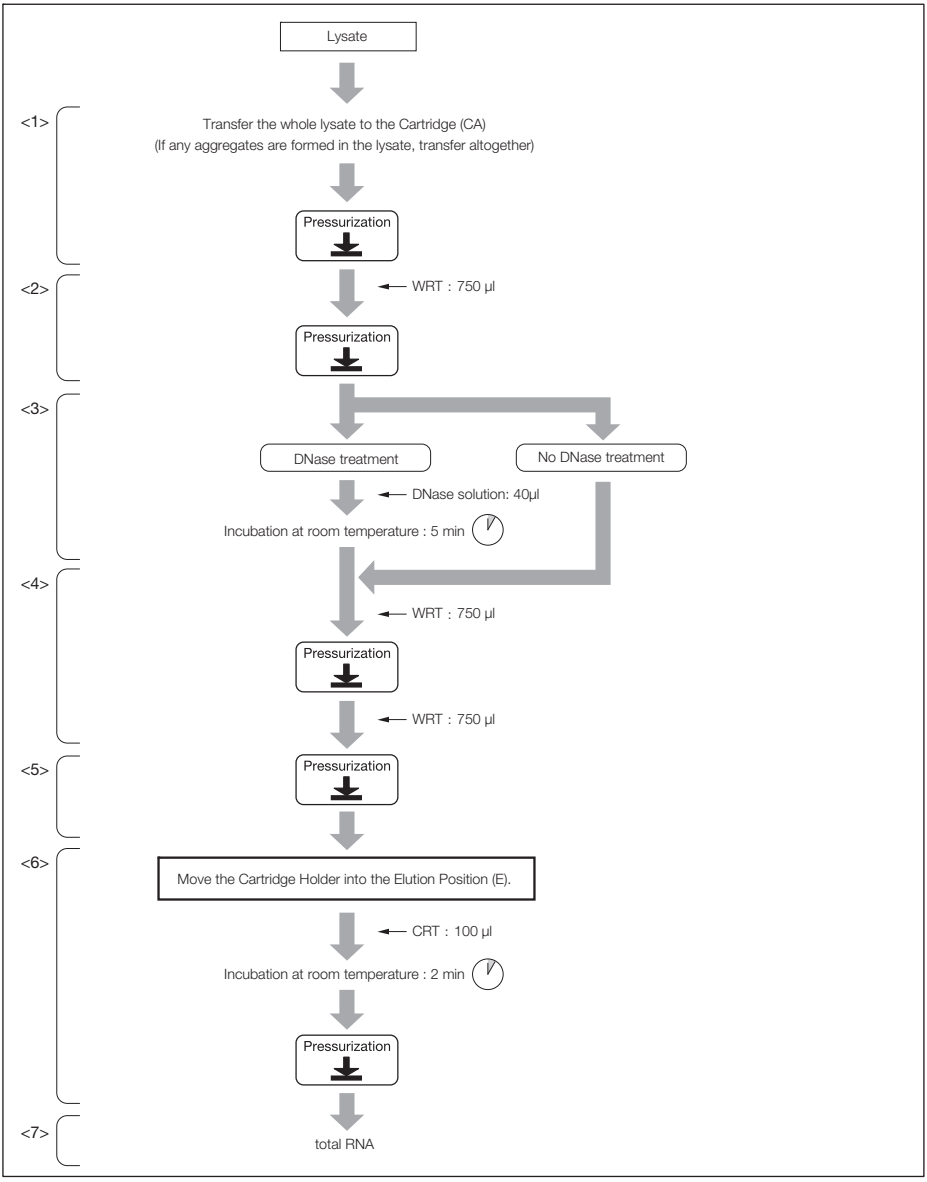
- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- If after repeated the lysate or WRT remain in any Cartridges (CA), the Cartridges should be removed, and the steps shown in Troubleshooting ((4) p.31) taken.

- Any solution and waste fluid containing LRT 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.



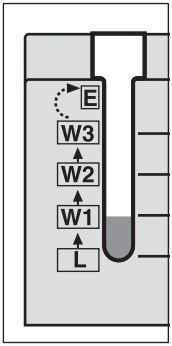
Details of QG-Mini80 Workflow

<1> <Applying Lysate> Carefully transfer the whole lysate prepared at 8-2 (p.13) to each Cartridge (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no lysate remains in the Cartridges and then return the Rotary Switch to the original position.

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, after adding >99% ethanol transfer altogether with the aggregates to the Cartridge.
Perform the extraction operation quickly after completion of lysis.
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 WRT 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 WRT remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WRT remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
After first wash, the waste fluid scale of the Tube Holder indicates [W1] position. (Refer to the following illustration)



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

<3-1> Prepare the recommended DNase

Product Name	Manufacturer	Cat. No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Life Technologies	AM2222	2	40 U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

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

Preparation 1)

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

Preparation 2)

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

Preparation 3)

2.7 Kunitz units/µl DNase *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

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.

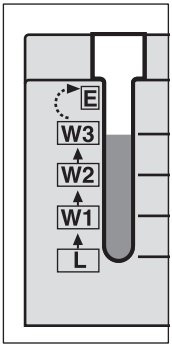
It is necessary to start DNase treatment after the first wash.
Avoid touching the filter in the Cartridge with the pipette tip during addition of DNase solution.
Pressurization should not be performed during incubation.
It is necessary to start pressurization after addition of WRT for the second wash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

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

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

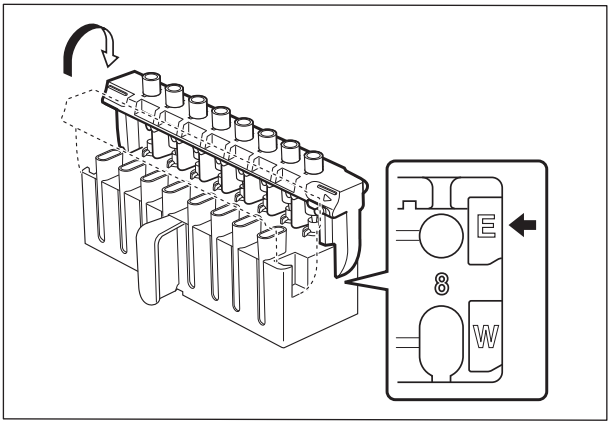
<5> <Third wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WRT to the Cartridges (CA). Set the Cartridge Holder and the Tube Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure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

Pressure application automatically stops in about 1 min. If any WRT 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 WRT 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 CRT to the Cartridges (CA) and then set the Cartridge Holder and the Tube Holder in QG-Mini80. After setting it, check the Wash Label cannot be seen by hiding under the Tube Holder.
After incubating at room temperature for 2 min, rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no CRT remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CRT remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
The volume of CRT may be reduced to 50 µl. If the volume is reduced, it is recommended to extend incubation time to 4 min.



<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 and then insert the Collection Tubes into the Tube Rack (optional) and then put Caps (CAP).
When the Tube Rack is not used, remove the Collection Tubes after putting Caps on them.
When using commercially available 1.5 ml microtubes : Put caps on 1.5 ml microtubes and then remove them.
Dispose the Waste Tubes and waste fluid according to appropriate laws and rules.

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

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA tissue kit S Ⅱ (RT-S2).

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

(1) Homogenization tube is ruptured (in the case of using a ball mill homogenizer) :

Cause	Action
Used at over speed	Follow the designated speed.
Not enough solution volume for homogenization	Add specified volume of LRT (500 µl) to the tube.
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
Inappropriate storage conditions for the tissue sample	Yield of RNA varies depending upon the type, bulkiness, amount, storage period, and storage conditions of each tissue. Do not use any sample once thawed. If not processing the tissues after collection, the tissue should be flash frozen in liquid nitrogen and stored at –80°C.
Inappropriate amount of tissue sample to be treated	Perform extraction within an appropriate amount of tissue (See Table 2 p.7).
Insufficient homogenization after addition of the LRT (2-ME added)	Homogenize completely according to instructions in section 8-2 step <3> (p.15 and 18). If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5 mm ϕ) is placed in the tube.
Not using appropriate protocol for the weight of your tissue sample	Select the correct protocol for the amount tissue (15 to 30 mg : p.14, 5 to 15 mg : p.17).
No addition of 2-ME to LRT	Dispense a required volume of LRT before use, and add 10 µl of 2-Mercaptoethanol (2-ME) per 1 ml of LRT.
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.34.
No addition of the prescribed volume of SRT or ethanol	Add the prescribed volume of SRT or >99% ethanol. If any sample is lost during homogenization, adjust the volumes of SRT and >99% ethanol.
Inappropriate addition order of reagents	Add SRT to the homogenate, vortex, and then add >99% ethanol.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See 8-1 p.12).
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.
Failure to reset the QG-810 “ELUT DIP TM” parameter or the QG-800 “CLCT DIP TM” parameter (*)	Confirm the parameters have been changed, particularly that the setting of the parameter of “ELUT DIP TM” (QG-810) is “120” or “CLCT DIP TM” (QG-800) is “240” (See Appendix 1 p.36, Appendix 2 p.38).

Cause	Action
Insufficient volume of CRT	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 “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 CRT is 100 µl.
Perform pressurization without adding WRT to the Cartridge (CA) after incubation for 5 min following the addition of a DNase solution (**)	Add the DNase solution, incubate for 5 min, then add WRT to the Cartridge before pressurization, and perform centrifugal operations.
No incubation at the time of elution (**)	After adding CRT onto the filter, and then incubate for 2min.
CRT volume changed to 50 µl	When reducing CRT volume to 50 µl, it is recommended that the incubation time be elongated to 4 min. In the case of QG-810, it is recommended to change the parameter “ELUT DIP TM” is “240” (See Appendix 1 p.36).
Room temperature too high or too low	All steps of the protocol should be done within the prescribed temperature range (15-28°C).
Use of reagents other than CRT to elute RNA	Use CRT to elute RNA.
Leaving Cartridges (CA) after lysate or WRT are discharged (**)	Once extraction with the QG-Mini80 has been started, work quickly without interruption. If an interruption has occurred, recovery may be improved the incubation time of elution to 4 min.
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)	Make sure to add specified volume of DNase reaction buffer to DNase solution.
Rupturing of filter when adding 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.
RNA degradation	Refer to (5) “RNA degradation”.

(3) Purity of RNA is low :

Cause	Action
No use of prescribed washing conditions	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.36-40). QG-800 : Wash the filter three times with 750 µl of WRT.
Foaming has occurred during addition of lysate to Cartridge (CA)	To avoid foaming, pipette lysate gently.
Inappropriate storage conditions for the tissue	Yield of RNA recovered can vary depending on the type, size, amount, storage period and storage conditions of the tissue specimens. Do not use specimens once thawed.
Insufficient homogenization after addition of LRT (2-ME added)	Homogenize completely according to 8-2 step <3> (p.15 and 18). If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5mm ϕ) is placed in the tube.

Cause	Action
No addition of the prescribed volume of SRT and >99% ethanol when preparing the lysate	Add the prescribed volume of SRT or >99% ethanol. If loss has occurred during homogenization, adjust the volume of SRT and/or >99% ethanol corresponding to the volume of the homogenate.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See 8-1 p.12).
Use of reagents other than CRT to elute RNA	Use CRT to elute RNA.

(4) Clogging of Cartridge (CA) occurs :

Cause	Action
Not using appropriate protocol for the amount of tissue	Select the correct protocol for the weight of tissue (15 to 30 mg : p.14, 5 to 15 mg : p.17). If a tissue tends to cause clogging, try using the protocol for samples weighing 15 to 30 mg (p.14). If you use this kit for the first time, start with 10 mg of tissue. Performing a preliminary test is recommended.
Use of excessive amount of a tissue	Reduce the amount of tissue. See Table 3 (p.11). For example, actual size of 30 mg liver is as follows :  Top  Side
Insufficient homogenization after addition of LRT (2-ME added)	Homogenize completely according to instractions in step <3> (p.15 and 18). If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5mm ϕ) is placed in the tube. For example a state of a liver sample after homogenization is shown below. 
Transfer tissue debris with the supernatant after centrifugation following homogenization	Repeat centrifugation or prolong centrifugation time.
Insufficient pressurization (**)	Pressurize once more.
No addition of the prescribed volume of SRT or ethanol	Add the prescribed volume of SRT or >99% ethanol. If any sample is lost during homogenization, adjust volumes of SRT and >99% ethanol.
No addition of the prescribed volume of ethanol to WRT	Before using WRT for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.12).
Room temperature too high or too low	All steps of protocol should be done within the prescribed range of room temperature (15-28°C).
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.

Cause	Action
QG-810/QG-800 : Operation panel of "- (QG-810)" or "× (QG-800)" is displayed ,and lysate or WRT is remained even after pressurization (*) QG-Mini80 : Failure to remove lysate or WRT completely despite repeated pressurization (**)	Take a filter out of the clogged Cartridge (CA) and try the recovery of RNA according to "Further Note" p.34.

(5) RNA degradation :

Cause	Action
Inappropriate storage conditions for tissue sample	If tissue samples are not immediately proceed, they should be flash frozen in liquid nitrogen and stored at –80°C or below. Do not use any sample once it has thawed.
No addition of 2-ME to LRT	Dispense the required volume of LRT before use, and add 10 µl of 2-Mercaptoethanol (2-ME) per 1 ml of LRT.
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 avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of recommended RNase-free DNase. For the details, inquire to each manufacturer.
Tissue has been left standing at room temperature after addition of LRT (2-ME added)	Start homogenization immediately after addition of the LRT.
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 insuficient.
RNA degradation	See (5) "RNA degradation".
No use of prescribed washing conditions	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.36-40). QG-Mini80 : Wash the filter three times with 750 µl of WRT.

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

Cause	Action
Use of unrecommended DNase	Only use one of the recommended DNases (See 3-[1] p.4).
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). In case 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 CRT or no operation of discharging (*)	Set the prescribed volume of CRT according to Table 4 (p.12). In addition, it is necessary to perform a discharging operation according to the User’s Guide of QG-810/QG-800.
No addition of CRT (**)	After insert the Cartridge Holder to the Elution Position (E), add 100 µl of CRT to Cartridge (CA).
No transfer of Cartridge Holder to the Elution Position (E) when adding CRT (**)	When adding CRT, addition has to be started after the transfer of the Cartridge Holder to 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 a Cartridge to a new Cartridge, and perform the operations from 8-3 <1> (p.20) again.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

b) If WRT remain in Cartridge (CA) :

Discard WRT remaining in Cartridge.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

In case of QG-Mini80

a) If clogging occurs at the lysate pressurization step :

Transfer the lysate remaining in a Cartridge to a new Cartridge, and perform the operations from 8-4 <1> (p.25) again.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

b) If clogging occurs at the lysate washing step :

Discard WRT remaining in Cartridge.
For the recovery of RNA from a clogged filter in Cartridge, see the procedures from 1) described below.

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

1) Dispense 350 µl of LRT (2-ME added) to a 1.5 ml microtube in advance.
2) Prepare tip-curved, acuminate tweezers or a tweezers for otolaryngologic use.
   Be careful not to contaminate with RNase by baking the tip of tweezers with burner flame or wiping with a RNase remover.
3) By reference to Figures 2 and 3, dismount filter from Cartridge (CA) with pushing rim of filter with the tip of tweezers.
4) Soak the dismounted filter into LRT (2-ME added) placed in 1.5 ml microtube, which has been prepared 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 drops from the inside of the lid.
6) Take out the filter, and put it into another tube (after completion of recovery, discard the filter).
7) Proceed to 8-2 <5> (p.19) to recover total RNA according to the method for extracting total RNA from 5 to 15 mg of tissue.

Figure 2 Appearance of a tweezers put into Cartridge (CA)

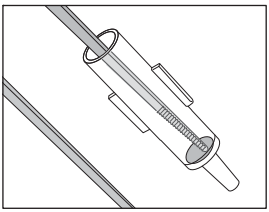
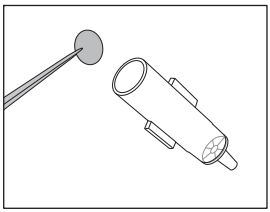


Figure3 Appearance of a filter dismounted



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*	

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Appendix 1 Setting of QG-810 Parameter

In the case of using QG-810, the parameter of “RNA TISSUE PLUS” and “RNA TISSUE” is the following table.
For total RNA extraction using this kit, the “ELUT DIP TM” parameter of the “RNA TISSUE PLUS” and “RNA TISSUE” modes need to be changed.
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 TISSUE PLUS (with DNase treatment)			RNA TISSUE (without DNase treatment)		
		PARAMETER	Check	Mode dafault	PARAMETER	Check	Mode dafault
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		750	750		750
5	WASH VOL2	750		750	750		750
6	WASH VOL3	750		750	750		750
7	WASH VOL4	750		750	750		750
8	WASH VOL5	750		750	750		750
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		750	750		750
14	WAS2 VOL2	750		750	750		750
15	WAS2 VOL3	750		750	750		750
16	WAS2 VOL4	750		750	750		750
17	WAS2 VOL5	750		750	750		750
18	ELUT VOL	100		100	100		100
19	ELUT PEAK	100		100	100		100
20	ELUT DIP TM	120		30	120		30

If the volume of CRT needs to be changed to 50 µl, the “ELUT VOL” parameter should be set at “50”. At that time, it is recommended that the “ELUT DIP TM” parameter be reset to “240”.

<How to change parameters>

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

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

   SETUP START
   ↓
   RNA TISSUE
   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.
   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 DIP TM” change to “120” :
   “ELUT DIP TM” appears by [MODE] button → change from “30” to “120”

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

   SETUP WRITING
   Save setting content.
   ↓
   SETUP COMPLETED
   ↓
   RNA TISSUE
   Returns to Ready status.

Appendix 2 Setting of QG-800 Parameter

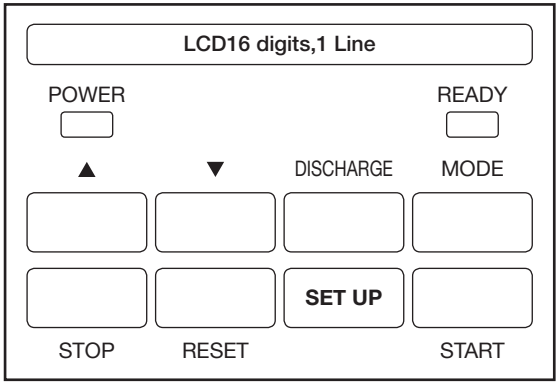
In the case of using QG-800 the parameter of “RNA PLUS” and “RNA” is the following Table.
It is also possible to use the “ISOLATE A” and “ISOLATE B” modes with this kit.
Parameters can be changed as shown in the table below.
When changing the parameter, refer to QG-800 User’s Guide.

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

Display Sequence	Operation menu	RNA PLUS (with DNase treatment)				RNA (without DNase treatment)			
		PARAMETER	Check	“RNA PLUS” mode dafault	“ISOLATE B” mode dafault	PARAMETER	Check	“RNA” mode dafault	“ISOLATE A” mode dafault
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	100		100	200	100		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

<How to change parameters>

1. Switching to “MAINTE MODE”
   1) Turn power on while pressing [START] and [▼] buttons simultaneously.
   2) Release the [START] and [▼] buttons after the operation panel displays “TP MODE.”
   3) When the operation panel displays “TPOO : SENSOR TEST”, press the [▲] or [▼] buttons to change the initial “O” into “F.” Then, press the [MODE] button, to change the next “0” 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 the [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 on. Press the [MODE] button to display the mode requiring changes to the 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 second later, the current mode (e.g., “ISOLATE A”) is displayed. About another second later, the first of the operating parameters and its current value are displayed. “✻” at the end indicates the current value.



2. Press the [MODE] button the necessary number of times to display the operating parameter to be changed.
   The [DISCHARGE] button is pressed to return to the previous parameter.
   After the last parameter, the display returns to the first parameter.
   Change the parameter to the value listed in the “PARAMETER” shown in the table (p.38).
3. The parameter value is changed by pressing [▲] or [▼] button.
   Pressing [▲] button increases the parameter value, while pressing [▼] button reduces the parameter value.
   If either the [▲] or [▼] button is kept depressed, the value will change serially.
   An attempt to increase the value from the maximum value will cause the parameter to be set at the minimum value. An attempt to reduce the level from the minimum value will cause the parameter to be set at the maximum value.
4. If other parameters need to be reset, repeat steps 2 and 3.

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

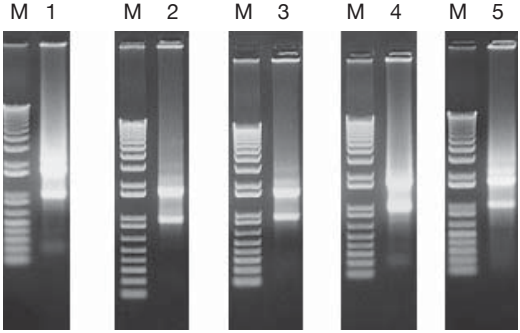
6. Return to “USER MODE”
   1) Turn power off.
   2) Follow steps 1-1) through 1-4).
   3) Press the [START] button to display “MENU : MAINTE MODE.” Then, press the [▼] button to display “MENU USER MODE.”
   4) While “MENU USER MODE” is displayed, press the [RESET] button and [SET UP] button (the unnamed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

Appendix 3 Examples of the Data with QuickGene RNA tissue kit S Ⅱ (RT-S2)

● **Results of electrophoresis (non denaturing gel electrophoresis)**

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

Figure 3



No.	Sample (with DNase treatment)
1	Liver
2	Brain
3	Lung
4	Kidney
5	Spleen

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

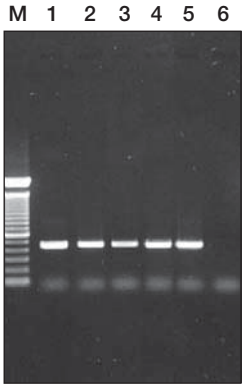
● **RT-PCR**

Figure 4 illustrates the result of RT-PCR amplification using diluted total RNA extracted with this kit. RT-PCR was performed G3PDH mRNA with following condition.

<RT condition>
Template : total RNA 500 ng
Enzyme : SuperScript Ⅱ
(Life Technologies)

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

Figure 4



No.	Sample (with DNase treatment)
1	Liver
2	Brain
3	Lung
4	Kidney
5	Spleen
6	Negative Control

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

RT-PCR amplification for G3PDH was successfully performed using cDNA corresponding to 10 pg/μl total RNA.

KURABO

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RT-S2_HB-E_V31