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HANDBOOK

QuickGene RNA tissue kit S
(RT-S)

For Isolation of total RNA from tissue samples

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Warning: For research use only.
Not recommended and intended for diagnostic or clinical application for human and animals.

1. Introduction

Fuji Photo Film Co., LTD developed and patented an evolutionary, porous membrane to immobilize nucleic acid. Because of its large specific surface area and uniform & fine porousness, QuickGene successfully isolates 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 QuickGene RNA tissue kit S is used with the QuickGene-series Automatic Nucleic Acid Isolation System, high quality and high yield total RNA can be isolated and also purified from tissue samples. In addition, total RNA from 8 sets of tissue lysate samples can be simultaneously extracted in only 13 minutes, without using not only spin columns but also hazardous solvent such as phenol. The purified, high quality total RNA is suitable for RT-PCR, northern blot analysis and other applications.

Please read this handbook carefully before using the kit.

2. Components of the kit

The kit includes the reagents necessary for 96 sets of total RNA isolation.

- ☐ Lysis buffer (LRT)
- ☐ Solubilization buffer (SRT)
- ☐ Wash buffer (WRT)
- ☐ Elution buffer (CRT)
- ☐ Cartridges (CA)
- ☐ Collection tubes (CT)
- ☐ Caps (CAP)
- ☐ Waste tubes (WT)

3. Storage conditions

Store all reagents at 15°C to 28°C.

4. Other required materials, not supplied in this kit

◆ **Reagents**

- >99% Ethanol
- 2-mercaptoethanol (2-ME)
- RNase free PBS
- DNase [For optional process. Recommended products are listed as below.]

RQ1 RNase-Free DNase	(Promega: Cat. No. M6101)
DNase I, Amplification Grade	(Invitrogen: Cat. No. 18068-015)
DNase I, Amplification Grade	(Sigma: Cat. No. AMP-D1)
Deoxyribonuclease (RT Grade)	(Nippon Gene: Cat. No. 313-03161)
DNase I, RNase-Free	(Ambion: Cat. No. 2222)
RNase-Free DNase Set	(QIAGEN: Cat. No. 79254)

◆ **Instruments and equipments**

- QuickGene-series Automatic Nucleic Acid Isolation System
- 1.5 ml Micro-centrifuge tubes
- Centrifuge tubes (see Table1)
- Micropipettes and tips
- Homogenizer
    - a. Rotor-stator homogenizer
    - b. Homogenization Pestle for micro- centrifuge tube
    - c. Beads mill homogenizer
- Vortex mixer
- Micro-centrifuge
- Tube stands

Table1 Recommended centrifuge tubes.

Size of QuickGene-series centrifuge-tube holder	Type of centrifuge tube	Product name (Examples)
Standard	Large centrifuge tube (for WRT)	BD Falcon™ 50 ml conical tube
	Small centrifuge tube (for CRT)	BD Falcon™ 15 ml conical tube
Large	Large centrifuge tube (for WRT)	BD Falcon™ 175 ml conical tube BD Falcon™ 225 ml conical tube
	Small centrifuge tube (for CRT)	BD Falcon™ 50 ml conical tube

Centrifuge tubes are used with the QuickGene-series Automatic Nucleic Acid Isolation System as containers for the Wash buffer (WRT) with ethanol and Elution buffer (CRT).

5. Safety warnings

Warning: For research use only.
Not recommended and intended for diagnostic or clinical application for human and animals.

- All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, gloves and safety glasses 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://lifescience.fujifilm.com)

Lysis Buffer (LRT)
Poisonous if swallowed
Don’t put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.
Wear laboratory coat, gloves and safety glasses during experiments.

Solubilization Buffer (SRT)
Don’t put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Wash Buffer (WRT)
Don’t put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

Elution Buffer (CRT)
Don’t put reagents in eyes and be careful of accidental ingestion.
In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

- Keep away the Lysis Buffer (LRT) from heat. Do not mix with disinfectants such as bleach.
- For disposal of waste fluid and consumables: When using potentially infectious samples for experiments, dispose them according to applicable regulations.

6. Precautions

- Refer to the MSDS (Material Safety Data Sheet) for specific recommendations on properties and handling. The MSDS can be obtained from the World Wide Website (http://lifescience.fujifilm.com).
- Refer to the user’s manual for the QuickGene-series Automatic Nucleic Acid Isolation System before using.

<Prevention against RNase contamination>

- Wear the disposable gloves when you have been handling the RNA and/or kit, because of prevention of RNase contamination.
- Use the disposable sterilization plastic materials during the operations. These materials are almost experience RNase free, but not guarantee, therefore, usually you may not need RNase free process.
- In case of using the glass or metal materials, you have to hot-air sterilize them at 200°C, more than 16 hr.

7. Quality controls

- The stability of the reagents is guaranteed for 9 months after purchase if stored at the specified temperature (15°C to 28°C).
- As part of the stringent of quality assurance program in Fuji Photo Film Co., LTD, the performance of QuickGene RNA tissue kit S is evaluated routinely on a lot-to-lot uniformity.
- QuickGene RNA tissue kit S is tested for contaminations of RNase.
- Quality and yield of isolated total RNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm), and RT-PCR amplification.

8. Protocols

8-1 Preparation of reagents

Lysis Buffer (LRT)

Mix thoroughly before using.
If the precipitates are contained in Lysis Buffer, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Lysis Buffer, cool down the bottle to room temperature before using.
Dispense the requirement volume and add 2-ME for 10 μl/1 ml LRT, each time.

Solubilization Buffer (SRT)

Mix thoroughly before using.
If the precipitates are contained in Solubilization Buffer, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Solubilization Buffer, cool down the bottle to room temperature before using.

Wash Buffer (WRT)

Provide the concentrated solution.
Add 40 ml of >99% ethanol into the bottle and mix with inversion the bottle gently at the beginning of use.

Requirements of Wash Buffer (WRT) with >99% ethanol and Elution Buffer (CRT)

Prepare the requirements of Wash Buffer (WRT) with >99% ethanol and Elution Buffer (CRT) according to the number of samples for isolation; refer to the following table.
Take some of the buffers into each tube and set the tubes in the QuickGene-series system tube holder. (See the user's manual of QuickGene-series Automatic Nucleic Acid Isolation System.)

Table 2 Buffer volume and the number of samples to set in the QuickGene System

Number of samples	WRT with 99% Ethanol	CRT
8	26 ml	9 ml
16	44 ml	11 ml
24	62 ml	13 ml
32	80 ml	15 ml
40	99 ml	17 ml
48	117 ml	19 ml
56	135 ml	21 ml
64	154 ml	22 ml
72	172 ml	24 ml
80	190 ml	26 ml
88	209 ml	28 ml
96	227 ml	30 ml

8-2 Sample preparations

- Basically, the QuickGene RNA tissue kit S is designed for total RNA isolation from 5 mg of mammalian tissue sample. However several tissue samples would be able to isolate the total RNA from more than 5 mg. (See Table 3)

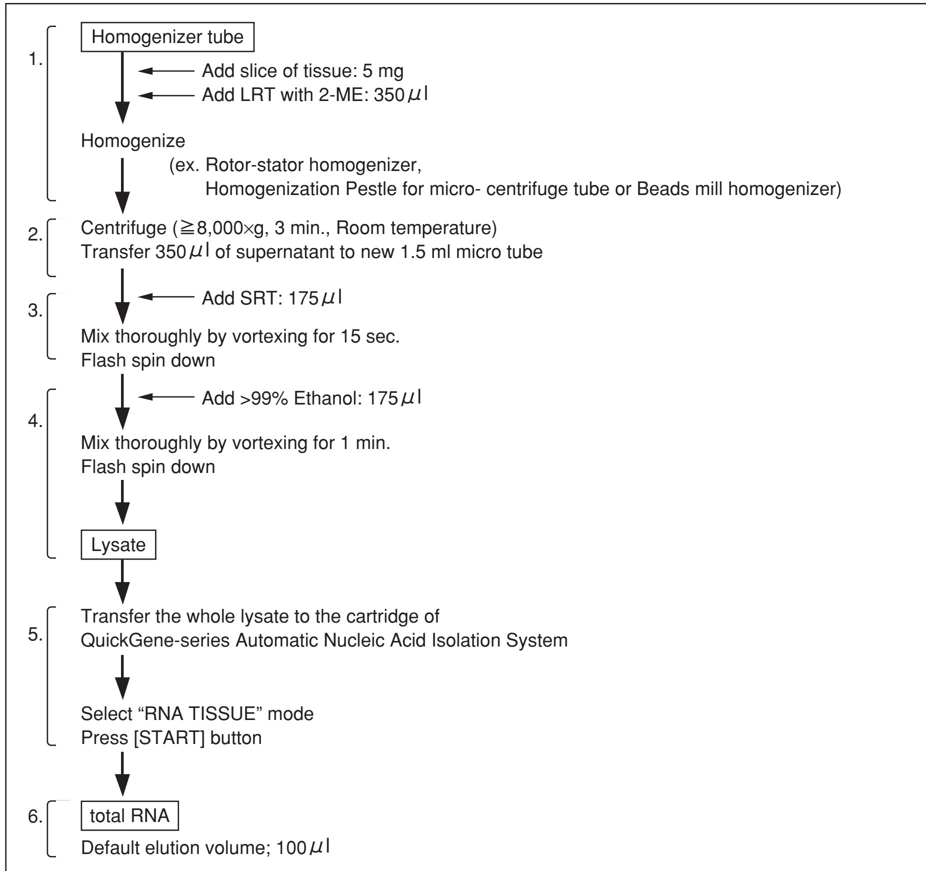
Table 3 Average yield and maximum sample amount of starting material for mouse tissue

Mouse Tissue	Average yield* (sample amount)	Maximum sample amount*
Liver	20 μg (5 mg)	30 mg
Brain	12 μg (30 mg)	50 mg
Spleen	15 μg (10 mg)	30 mg
Kidney	15 μg (10 mg)	20 mg
Lung	12 μg (20 mg)	30 mg
Heart	10 μg (15 mg)	20 mg
Thymus	15 μg (10 mg)	20 mg

* Completely homogenize with using the beads mill homogenizer. The yield depends on the sample type and the storage condition. These yield are typically data of mouse tissues.

- Tissue type, tissue storage condition and homogenize method may change the maximum sample amount and extend the processing time. If the cartridges are clogged, reduce the amount of sample.
- Soak the 5 mg of sliced tissue in the Lysis Buffer (LRT) and homogenize immediately after sample collection. If you do not prepare the samples immediately, freeze the tissue with liquid nitrogen and store at -80°C.
- Keeping the samples at room temperature for a long time and/or thawing tissue during handling may degrade RNA or lower the yield.
- Use calibrated pipets for the buffer dispensing. The volumes are adjusted for the best performance of the system.
- Take all of operation rapidly at room temperature (15-30°C).

<Preparation workflow “RNA TISSUE” mode: without DNase treatment>



Details

1. The membrane will be clogged, when excessive amounts of tissue sample applied to cartridge. Use LRT with added 2-ME. Please add 2-ME (10μl/1 ml LRT) to LRT before using.
   When using the frozen sample, keep them frozen just before homogenize.

   Recommended homogenizers are follows three. Please read the user's manual of homogenizer before the operation.
   The maximum sample volume using the Homogenization Pestle for micro- centrifuge tube, may become smaller than using rotor-stator homogenizer or beads mill homogenizer.
   a. Rotor-stator homogenizer
      Put tissue sample and 350μl of LRT with 2-ME to a fresh micro tube, and immediately homogenize with Rotor-stator homogenizer until the sample is uniformly homogeneous. Please optimize the rotation speed and time for your sample.
   b. Homogenization Pestle for micro- centrifuge tube
      Use the exclusive motor and place the Pestle to the motor.
      Put tissue sample and 200μl of LRT with 2-ME to a fresh micro tube, and immediately homogenize with Pestle until the sample is uniformly homogeneous. Add 150μl of LRT in homogenate and mix thoroughly by vortexing with maximum speed for 15 sec. Flash spin down the homogenate.
   c. Beads mill homogenizer
      Put tissue sample and 350μl of LRT with 2-ME to a fresh micro tube, and immediately homogenize with Beads mill homogenizer until the sample is uniformly homogenous.

2. Centrifuge (≧8,000×g, 3 min.) at room temperature to remove insoluble materials and transfer the 350μl of supernatant to a fresh micro tube. It may be necessary to increasing the rotation speed and time, in order to spin down insoluble materials completely.

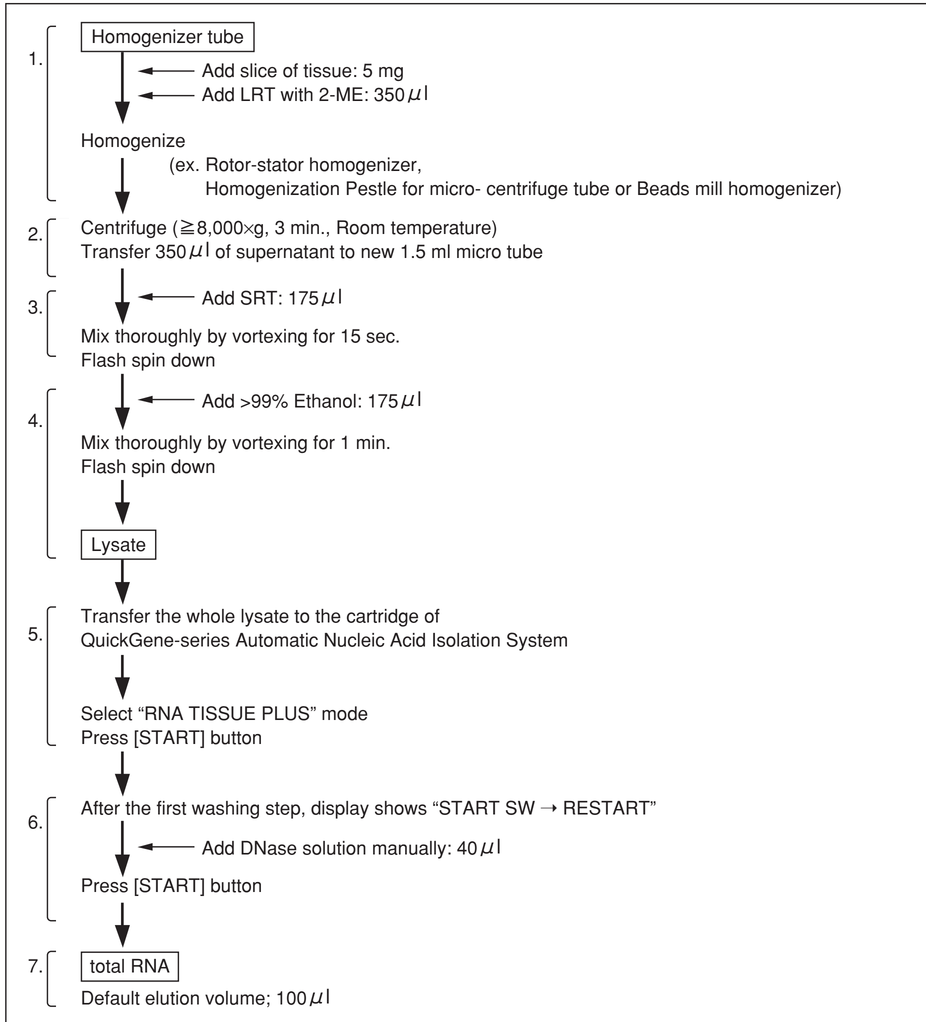
3. Add 175μl of SRT and mix thoroughly by vortexing with maximum speed for 15 sec., and flash spin down.

4. Add 175μl of >99% Ethanol and mix thoroughly by vortexing with maximum speed for 1 min., and flash spin down the lysate completely. Incomplete vortexing may cause low yield.

5. Transfer the whole lysate to the cartridge of QuickGene-series Automatic Nucleic Acid Isolation System and immediately press the start button. If any aggregates are present in the lysate in step 4, apply all of them with the lysate to the cartridge.

6. Default elution volume is 100μl but you may change the setting of elution volume less than default volume, minimum 50μl. In case of setting to 50μl, yield may decline.
   If you do not use the eluted total RNA for any experiments immediately, after covering the Caps (CAP) on the Collection Tubes (CT) tightly, store at -20°C or -80°C.

<Preparation workflow “RNA TISSUE PLUS” mode: with DNase treatment>



Details

1-5. Refer to < Preparation workflow “RNA TISSUE” mode: without DNase treatment >

6. Add the recommended DNase manually after the first washing step, display shows “START SW → RESTART”.

6-1. Prepare the recommended DNase

Product Name	Manufacturer	Cat. No	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40μl
DNase I, Amplification Grade	Invitrogen	18068-015		
DNase I, Amplification Grade	Sigma	AMP-D1		
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Ambion	2222	2	40 U/40μl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4 Kunitz unit/40μl

*1; Dissolve 1,500 Kunits units of DNase I with 550μl of RNase-Free water before preparing the DNase I reaction solution.

Preparation 1

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

Preparation 2

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

Preparation 3

2.7 Kunitz unit/μl DNase I	1.25μl
Buffer RDD	35μl
RNase-Free water	3.75μl

6-2. Addition of DNase I
Open the front cover of the instrument and add 40μl of DNase I reaction solution to each cartridge by using micropipettes. Do not touch the membrane by pipet tip when DNase I reaction solution is added. Close the front cover and hold for 5 min.

6-3. Default waiting (holding) time of DNase treatment is 5 min. You may change the setting of time as the parameter of a program.

7. Default elution volume is 100μl but you may change the setting of elution volume less than default volume, minimum 50μl. In case of setting to 50μl, yield may decline.
If you do not use the eluted total RNA for any experiments immediately, after covering the Caps (CAP) on the Collection Tubes (CT) tightly, store at -20°C or -80°C.

8-3 total RNA isolation using the QuickGene-series Automatic Nucleic Acid Isolation System

Notice: System set up and basic operations
Please read the user's manual of QuickGene-series Automatic Nucleic Acid Isolation System circumstantially for the details before using the system.

(1) Selection of isolation mode

Select "RNA TISSUE" or "RNA TISSUE PLUS" mode for total RNA isolation from tissue with the kit.

(2) Setting of cartridges and tubes

Open the front cover of the instrument and set the collection and waste tubes in the collection-tube holder.

- Use the specified Collection Tubes (CT) and Waste Tubes (WT) including in the kit.

Attach the cartridge holder to the instrument and set 1~8 cartridges in the cartridge holder.

- Use the specified Cartridges (CA).

Notice: Refer to the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System for details of setting cartridges and tubes.
Incorrect cartridge placement may result in the solution spilling or improper isolation.
Wear gloves during the experiments to avoid nuclease contamination.

(3) Setting of reagents

Prepare the required volume (see 8-1 Preparation of reagents) of Wash Buffer (WRT) with >99% ethanol and Elution Buffer (CRT) into the tubes; set them to the holder; and put the holder to the designated positions of instrument.

Notice: Wear gloves during the handling of reagents to avoid nuclease contamination.

- Read the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System for details for setting reagents.

(4) Discharge

Set the "discharge tray" and check the collection holder and cartridge holder setting for the correct positions.
Press the "DISCHARGE" after closed the front cover of the instrument.

Notice: Because of air in the tubings, incorrect volume of reagents may occur without discharge operation.

(5) Applying the prepared samples

Apply all contents of prepared lysate samples (see 8-2 Sample preparations) into the each Cartridge (CA) by using micropipettes (any aggregates in the lysate should be transfered into the cartridge).

(6) Isolation

Close the front cover of the instrument.
Confirm the appropriate mode on the operation panel and press the [START] button.
When using the 30 mg of mouse liver, elution time may extend until 25 min. or the cartridge on QuickGene-series may clog. In this case, reduce the tissue sample volume.

(7) Collection of total RNA

After completing the process, each sample result is indicated on the operation panel as follow;
v (Check): Completed normally
– (Hyphen): Not completed normally
_ (Underscore): No cartridge or no sample

Open the front cover and remove the Collection Tube(s) (CT) from the collection-tube holder.

- As total RNA is eluted from the Cartridge(s) (CA) using 100 μl of Elution Buffer (CRT), the volume of recovered total RNA solution will be 100 μl.

Cover with the Caps (CAP) on the Collection Tubes (CT) containing the isolated total RNA tightly.

(8) Clean up

Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.
Remove the cartridge holder and dispose the Cartridges (CA).

Warning: Disposal of waste fluid and consumables
When using the potentially infectious samples for experiments, dispose them according to applicable regulations.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene RNA tissue kit S. For system-related problems (e.g., when an error message appears), see the QuickGene-series user's manual.

(1) Low yield or no RNA obtained

Cause	Possible Solution
Improper storage condition of sample	Optimize storage conditions in different sample volume, and storage period. Avoid use of tissue samples kept at room temperature for a long time and/or thawing during handling.
Insufficient amount of sample was used	Increase the amount of tissue sample. (See Table 3)
Insufficient homogenization following the addition of Lysis Buffer (LRT) containing 2-ME	Allow sufficient homogenization until the sample is uniformly homogeneous, immediately after Lysis Buffer (LRT) addition.
Required volume of ethanol was added to Wash Buffer (WRT)	Make sure to add required volume of ethanol to the Wash Buffer (WRT) prior to use.
Use of the old Wash Buffer (WRT including ethanol)	Flash remaining Wash Buffer (WRT: including ethanol) which may be one day old or more in the instrument prior to use.
Lysate not fully applied to Cartridge(s) (CA)	If aggregates are present in the lysate, apply them with the lysate to the cartridge.
Insufficient amounts of reagents used	Make sure that sufficient amount of reagent are in the reagent bottles.
Insufficient volume of DNase reaction buffer has been added (for isolation with DNase treatment)	Make sure to add specified volume of DNase reaction buffer to DNase solution.
The membrane is damaged when DNase solution is added (for isolation with DNase treatment)	Avoid physical contact to the membrane when DNase solution is added.
RNA has been degradated	See (4) section

(2) Low purity (A260/A280)

Cause	Possible Solution
Excess amount of sample was used	Reduce the amount of tissue sample to below the specified amount. (See Table 3)

(3) Clogging the cartridge

Cause	Possible Solution
Insufficient homogenization following the addition of Lysis Buffer (LRT) containing 2-ME	Allow sufficient homogenization until the sample is uniformly homogeneous, immediately after Lysis Buffer (LRT) addition.
Excess amount of sample was used	Reduce the amount of tissue sample to below the specified amount. (See Table 3)

(4) RNA degradation

Cause	Possible Solution
Improper tissue sample storage conditions	Quickly freeze the tissue in liquid nitrogen that will not be used immediately, then store them at -80°C or below. Avoid use of tissue samples kept at room temperature for a long time and/or thawing during handling.
RNase contamination	Although all buffers, cartridges, and collection tubes are supplied RNase-free, RNase contamination may occur during preparation and storage. Extreme caution to avoid RNase contamination.
RNase contamination in DNase (for isolation with DNase treatment)	Use a recommended RNase-free DNase.
Heating of RNA	RNA would be degraded when heated. Store RNA samples on ice during experiments.

(5) Subsequent experiments (e.g., RT-PCR) unsuccessful

Cause	Possible Solution
Improper amount of RNA was used	Determine the RNA concentration based on the absorbance at 260 nm.
Contamination of genomic DNA	Isolate the total RNA by DNase treatment (“RNA PLUS” mode).
RNA has been degradated	See (4) section

(6) Incomplete of DNA degradation <“RNA PLUS” mode>

Cause	Possible Solution
Membrane not completely soaked in DNase solution	Make sure that DNase is evenly distributed over the membrane in the Cartridge(s) when DNase solution is applied.
Insufficient DNase activity	Make sure to use recommended volume of the DNase to have sufficient activity.

(7) Supplying the precipitates in reagents

Cause	Possible Solution
Stored at low temperature	Store solutions at 15°C to 28°C. If the precipitates are contained, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved.

(8) The collection tubes are empty after the elution.

Cause	Possible Solution
Missed the discharge	Set the “discharge tray” and check the collection holder and cartridge holder setting up into correct positions. Press the “DISCHARGE” after closed the front cover of the instrument. See the QuickGene-series user’s manual.

10. Ordering Information

Product	Cat #
QuickGene-series Automatic Nucleic Acid Isolation Systems	
QuickGene DNA tissue kit S	DT-S
Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from the tissue	
QuickGene DNA whole blood kit S	DB-S
Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from whole blood	
QuickGene RNA tissue kit S	RT-S
Dedicated reagent kit for QuickGene-series to purify the total RNA from the tissue	
QuickGene RNA cultured cell kit S	RC-S
Dedicated reagent kit for QuickGene-series to purify the total RNA from cultured cell	
QuickGene Plasmid kit S	PL-S
Dedicated reagent kit for QuickGene-series to extract the Plasmid DNA	

Trade Mark; FalconTM ™ (Becton, Dickinson and Company)
The Polymerase Chain reaction (PCR) is covered by patents owned by Roche Molecular Systems and F Hoffmann-La Roche Ltd.

11. Contact Information

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Appendix 1 "RNA TISSUE" mode and "RNA TISSUE PLUS" mode are set in the following parameter.

	RNA TISSUE	RNA TISSUE PLUS
PARAMETER	SET VALUE	SET VALUE
BIND PEAK	120	120
WASH COUNT	3	1
WASH PEAK	110	110
WASH VOL1	750	750
WASH VOL2	750	750
WASH VOL3	750	750
WASH VOL4	750	750
WASH VOL5	750	750
WASH DIP TM	150	150
WAS2 WAIT T	0	5
WAS2 COUNT	0	2
WAS2 PEAK	110	110
WAS2 VOL1	750	750
WAS2 VOL2	750	750
WAS2 VOL3	750	750
WAS2 VOL4	750	750
WAS2 VOL5	750	750
ELUT VOL	100	100
ELUT PEAK	100	100
ELUT DIP TM	30	30