KURABO

HANDBOOK

QuickGene-AutoS RNA Tissue Kit (AS-RT)

For extraction of total RNA from tissue

Ver.1.0

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. QuickGene also uses pressured filtration technology, which enables producing new, compact and automatic instruments for rapid nucleic acid purification.

This is a ready-to-use prepacked reagent kit for the extraction process of QuickGene-Auto12S (QG-Auto12S) or QuickGene-Auto24S (QG-Auto24S).

- When using this kit with QG-Auto12 or QG-Auto24S, high quality and high yield total RNA can be extracted and also purified from 5-30 mg of animal tissue.
- RNA from animal tissue samples can be simultaneously extracted in following time.
  QG-Auto12S：about 30 min for 12 sets of animal tissue samples
  QG-Auto24S：about 30 min for 24 sets of animal tissue samples
- The purified, high quality total RNA is suitable for RT-PCR, Northern blotting and other applications.

Please be sure to read the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S carefully before using this kit.

2. Kit Components and Storage Conditions

2-1. Kit Components (48 Preps)

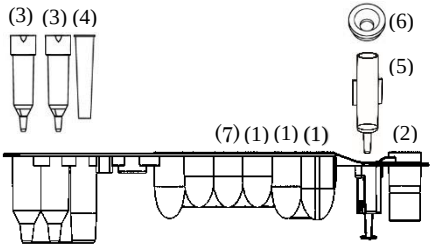
☐	Lysis Buffer LRT	42.5 ml
☐	Solubilization Buffer SRT	20 ml
☐	Reagent strip	48
☐	1 ml Long Tips	48
☐	Waste Tubes	48

2-2. Storage Conditions

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

2-3. Contents of reagent strips

☐	Wash Buffer	WRT	750 µl	3 positions	(1)
☐	Elution Buffer	CRT	250 µl		(2)
☐	Short Tip		2		(3)
☐	Tip pack		1		(4)
☐	Cartridge		1		(5)
☐	Pressure Adapter		1		(6)
☐	DNase solution *				(7)



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

  Recommended goods

  - RQ1 RNase-Free DNase (Promega: Cat. No. M6101)
  - Deoxyribonuclease (RT Grade) (NIPPON GENE: Cat. No. 313-03161)
  - DNaseI, RNase-Free (Thermo Fisher Scientific: Cat. No. AM2222)
  - RNase-Free DNase Set (QIAGEN: Cat. No. 79254)

[2] Equipment

- QuickGene-Auto12S or QuickGene-Auto24S
- Micropipette and tips
- 2 ml microtubes for samples

  Recommendation product: BM EQUIPMENT Cat. 4020

  SARSTEDT Cat.72.695.700, Cat.72.695.500S

  *When using a tube other than the recommended product, check the compatibility with the Collection holder beforehand.

- 1. 5 ml or 2 ml microtubes for elution of RNA
  Recommendation product: BM EQUIPMENT Cat. BM4015, Cat. BM4020
  SARSTEDT Cat. 72.706.700
  *When using a tube other than the recommended product, check the compatibility with the Collection holder beforehand.
- Tube stand
- Homogenizer:
  a. Ball mill homogenizer
  (KURABO PS-2000 / TOMY Micro Smash MS-100 / QIAGEN TissueLyser)
  b. Rotor-Stator homogenizer (KINEMATICA AG Polytron PT3100, etc.)
  c. Pestle homogenizer for microtube[*1]
  (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)[*2]
  QIAGEN TissueLyser : Treff Lab 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 5 mm φ) (For ball mill homogenizer only)
- Microcentrifuge (c.a. 17,000 × g (15,000 rpm))

*1 Please use a dedicated motor
*2 Sterilized tube is not recommended. Tube may be broken.

4. Safety Warnings

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

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

◆ 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.
- Caution should be exercised with respected to fire because it is a highly flammable liquid.
- Wear appropriate protective gloves and goggles when handling this chemical.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ 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.

◆ 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 of potentially infectious samples and consumables by incineration, high-temperature decontamination, sterilization, or disinfection in accordance with applicable laws. When entrusting waste disposal to licensed hazardous waste disposal contractors, use specially controlled waste management forms (manifest), if applicable.

◆ Use or storage of Reagent strips at the specified temperature (15^{o}C – 28^{o}C).

5. Precautions

◆Handling of Starting Material

- This kit corresponds to total RNA extraction from 5 to 30 mg of mammalian tissue sample.

Table 1. Maximum amounts of normal mouse tissue for each homogenizer

Balb/c mice (females, 7 weeks old).

Tissue	Ball-milling	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-AutoS RNA tissue kit (AS-RT) 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 1.
- Do not overload the cartridge, 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 specie, condition, type and homogenization condition. The amount of tissue that can be processed may be less than 30 mg.
- 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.

◆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

- All operations should be performed at room temperature (15-28°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Do not spend time during isolation and operate immediately.
- 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.
- Before starting operation, please make sure the following things:
  - Waste Tubes and 1.5 ml or 2 ml microtubes (for elution) are set in the Collection holder.
  - Reagent strips are set correctly in the Reagent holder.
  - 1 ml Long tips and 2 ml microtubes (Whole blood and EDB included) are set in the Reagent strip.
  - The lid of Reagent holder is completely closed.
  - Reagent holder and Collection holder are properly set in the holder guide.
- All operations should be performed at room temperature (15 to 28 °C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Except for unavoidable circumstances, please do not turn off the QG-Auto12S or QG-Auto24S device during operation. You cannot resume operation from the same process.
- Refer to the Operation Manual of QG-Auto12S / QG-Auto24S for 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-AutoS RNA Tissue Kit (AS-RT) is evaluated routinely on a lot-to-lot uniformity.
- Yield and quality of extracted total RNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

7. Product Description

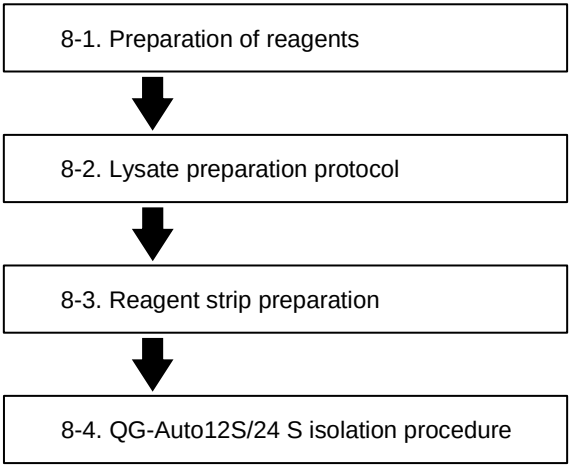
Table 2 shows the average of yield and purity (A260/280) of total RNA extracted from normal tissue of mice using this kit.
The yield varies depending upon sample conditions.

Table 2　Yield and Purity of total RNA

Sample	Amount of tissue	Yield (µg)	A260/280
Liver	7 mg	30.7	2.33

8. Protocol

〔Overview Flow Chart〕



8-1. Preparations of Reagents

◆ LRT

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 500 µl of LRT per 1 cartridge. Add 10 µl 2-ME per 1 ml of LRT. Dispense in a fume hood, wear appropriate protective equipment and adjust it in the draft.

◆ SRT

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.

◆ DNase solutions (when using a DNase treatment)

Prepare the DNase solution according to the following tables.
After preparation, add this DNase solution into the dedicated well of reagent strip (Refer to p5.)

<Prepare the recommended DNase solutions>

Product Name	Manufacturer	Cat.No.	Preparation	Final conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20U/40 µl
DNase I, Amplification Grade	Thermo Fisher Scientific	18068-015		
DNase I, Amplification Grade	Sigma-Aldrich	AMP-D1		

Deoxyribonuclease(RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Thermo Fisher Scientific	AM2222	2	40U/40 µl
RNase-Free DNase Set*1	QIAGEN	79254	3	3.4Kunitz units/4 µl

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

Preparation 1)

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

Preparation 2)

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

Preparation 3)

2.7Kunitz units / µl DNase I※2	1.25 µl
Buffer RDD	35 µl
Nuclease Free Water	3.75 µl

*2: The QIAGEN protocol may cause excess DNase activity. We recommend the method above.

8-2. Lysate Preparation Protocol

This kit is correspond to isolate total RNA from 5-30 mg of animal tissue.
[Important notes before starting]

- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow (p.13, 15).
- 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]

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

15-30 mg: p.13
5-15 mg: p.15

15-30 mg: Lysate preparation flow

⚠ Note: Be sure to follow the sequence of steps <1> to <5>.
If the order is changed, the desired yield may not be obtained.

- Wear appropriate protective equipment to reduce the risk of chemical injury and infection.
- Wear gloves to avoid nuclease contamination when using reagent strips or tubes.
- Refer to the operation manual of QG-Auto12S/QG-Auto24S for details.

<1>Prepare fresh or frozen tissue 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 1. If your material is not listed in Table 1, we recommend starting with no more than 10 mg and use the protocol for 5-15 mg (p.15).

<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.5). If using a ball mill homogenizer, put a zirconia ball (5 mmφ) 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>).
There are the following methods <3a> to <3c> for tissue disruption and homogenization. Refer to Table 1 (p.8) before starting the experiment because the maximum sample amount is different depending on the sample homogenizer. Please carefully read the instruction manual of each device and homogenize it.
When adding LRT to a frozen tube, be careful not to blow out the tube contents or break the tube.
<3a> Ball mill homogenizer
Put the weighed tissue sample into the appropriate tube (3-[2] p.4) into the bottom of the tube with a zirconia ball (5 mmφ) 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 4: Suggested homogenization conditions suitable for use with 15 to 30 mg of tissue

	KURABO PS-2000	Tomy Micro Smash MS-100	Qiagen TissueLyzer
Liver, Brain, Lung/ Kidney and spleen	3,800 rpm, 60 s × 2 times	3,800 rpm, 300 s	30 Hz 5 min × 2 times
Heart	3,800 rpm, 60 s × 2 times	3,800 rpm, 300 s × 3 times	

<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. 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 1 p.8). 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 1 (p.8).

<4> Centrifuge at ≧17,000 × g (≧15,000 rpm) at room temperature for 3 min 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.

Transfer all lysate into new 2.0 ml microtube (sample tube) and start isolation immediately.

5-15 mg: Lysate preparation flow

Note: Be sure to follow the sequence of steps <1> to <5>.
If the order is changed, the desired yield may not be obtained.

- Wear appropriate protective equipment to reduce the risk of chemical injury and infection.
- Wear gloves to avoid nuclease contamination when using reagent strips or tubes.
- Refer to the operation manual of QG-Auto12S/QG-Auto24S for details.

<1>Prepare fresh or frozen tissue 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 1. If your material is not listed in Table 1, we recommend starting with no more than 10 mg and use the protocol for 5-15 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.5). If using a ball mill homogenizer, put a zirconia ball (5 mmφ) 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>).
There are the following methods <3a> to <3c> for tissue disruption and homogenization. Refer to Table 1 (p.8) before starting the experiment because the maximum sample amount is different depending on the sample homogenizer. Please carefully read the instruction manual of each device and homogenize it.
When adding LRT to a frozen tube, be careful not to blow out the tube contents or break the tube.

<3a> Ball mill homogenizer

Put the weighed tissue sample into the appropriate tube (3-[2] p.5) into the bottom of the tube with a zirconia ball (5 mmφ) 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.5)

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

	KURABO PS-2000	Tomy Micro Smash MS-100	Qiagen TissueLyzer
Liver, Brain, Lung/ Kidney and spleen	3,800 rpm, 60 s × 2 times	3,800 rpm, 300 s	30 Hz 5 min × 2 times
Heart	3,800 rpm, 60 s × 2 times	3,800 rpm, 300 s × 3 times	

<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. 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 1 p.8). 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 1 (p.8).

<4> Centrifuge at ≧17,000 × g (≧15,000 rpm) at room temperature for 3 min 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.

Transfer all lysate into new 2.0 ml microtube (sample tube) and start isolation immediately.

8-3. Reagent Strip Preparation

- Wear gloves to avoid nuclease contamination when using reagent strips or tubes.
- Refer to the operation manual of QG-Auto12S/QG-Auto24S for details.

<1> Prepare the Collection holder and Reagent holder on the workbench.
<2> Load the waste tube and 1.5 ml or 2 ml microtube into the Collection holder.
<3> Remove the Reagent strips from the kit box, place it in the Reagent holder, and insert 1 ml Long Tip in the specified position (p.5 (4)).

8-4. QG-Auto12S/QG-Auto24S operation

- Please read the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for the details before using the device.
- To avoid contamination of nuclease, wear disposable gloves during preparation of Reagent strips and microtubes.

<1> Open the front door and put the Collection holder and Reagent holder to the specified positions on the machine.
<2> Turn on the device.
The device proceeds through a self-check and moves to the home position about all moving parts.
<3> At the Home screen, select the “RNA TISSUE”.
<4> Chose the elution volume.
<5> Make sure all the accessories has been putted in the system. Tick the check list then the “Next” button will show up.
<6> Press the “Next” button.
<7> Check the protocol information is correct, then press the “Start” button to proceed the isolation. Then processing will be started.
  - During the running step, the touch panel show the processing and remaining time.
  - Operation status can be confirmed by blinking process name (LYSIS, BINDING, WASH, ELUTE, FINISH).
  - Do not open the front door of the device while running. If you open the front door, please read the Operation manual of QG-Auto12S / QG-Auto24S and resume operation.
  - To pause, touch the "Pause" button on the operation panel. The end confirmation screen will be displayed, please press "Yes" to finish.
<8> After finishing the protocol, the beeper will call and the process name "FINISH" flashes on the operation panel.
After confirming that the device is completely stopped open the front door, take out the Reagent holder and the Collection holder.
Take out the elution tube from the Collection holder.
  - If you do not use total RNA immediately, please close the tube lid tightly and store at -20°C or -80°C.

9. Trouble-shooting

Review the information below to troubleshoot the experiments with QuickGene-AutoS RNA Tissue Kit (AS-RT).

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

Causes	Measure
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, 5mmφ).
Use of a tube other than the prescribed microtubes for homogenization	Use the microtube appropriate for homogenizer.

(2) Low yield or no RNA obtained

Causes	Measure
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 1, p.8).
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.13, 5 to 15 mg : p.15).
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.
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.

(3) Purity of RNA is low

Causes	Measure
Inappropriate storage conditions for the tissue	Purity 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.13 and 15). If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5mmφ) is placed in the tube.
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.

(4) Clogging of Cartridge occurs

Causes	Measure
Not using appropriate protocol for the amount of tissue	Select the correct protocol for the weight of tissue (15 to 30 mg : p.13, 5 to 15 mg : p.15). If a tissue tends to cause clogging, try using the protocol for samples weighing 15 to 30 mg (p.15). 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 1 (p.8). 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 instructions in step <3> (p.13 and 15). If using a ball mill homogenizer, check the settings of the homogenizer and ensure that a ball (zirconia, 5mmφ) 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.
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.

(5) RNA degradation

Causes	Measure
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

Causes	Measure
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	See (5) “RNA degradation”.

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

Causes	Measure
Use of unrecommended DNase	Only use one of the recommended DNases (See 3-[1] p.5).
Insufficient DNase activity	Use a recommended DNase activity.

10. Ordering Information

Product	Content	Cat #
QuickGene-AutoS DNA Blood Kit	48 preps	AS-DB
QuickGene-AutoS DNA Tissue Kit	48 preps	AS-DT
QuickGene-AutoS plasmid Kit	48 preps	AS-PL
QuickGene-AutoS RNA Blood Kit	48 preps	AS-RB
QuickGene-AutoS Tissue RNA Kits	48 preps	AS-RT
QuickGene-AutoS RNA Cultured Cell Kit	48 preps	AS-RC

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