
HANDBOOK

QuickGene-AutoS RNA Virus Kit (AS-RV)

Contents

1. Introduction	4
2. Kit Components and Storage Conditions	4
2 - 1. Kit Components (48 Preps)	4
2 - 2. Storage Conditions	4
2 - 3. Reagent Strip Components	5
3. Other Required Materials, Not Supplied in This Kit	6
4. Safety Warnings	7
5. Precautions	8
6. Quality Control	9
7. Protocol	10
7-1. Preparations of Reagents	10
7-2. Lysate Preparation Protocol	10
7-2-1. Protocol for RNA Extraction from Culture Cell Supernatant Fluid	10
7-2-2. Protocol for RNA Extraction from Swab (nasopharynx, nasal cavity, or pharynx)	10
7-3. Reagent Strip Preparation	12
7-4. QG-Auto12S/24S Operation	12
8. Trouble-shooting	14
9. Ordering Information	15

Warning

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

1. Introduction

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

Features of this kit are as follows.

- Some reagents are individually enclosed for each sample.
- By using this kit with QG-Auto12S or QG-Auto24S, total RNA can be extracted from samples of viral infection such as swab (nasopharynx, nasal cavity, or pharynx), culture cell supernatant liquid, serum, plasma and saliva.
- Extraction time is as follows.
 - QG-Auto12S: Approx. 25 minutes *12 samples are processed in one time.
 - QG-Auto24S: Approx. 25 minutes *24 samples are processed in one time.
- Total RNA which does not include proteins and chaotropic salts is extracted. Extracted total RNA is suitable for application such as RT-PCR.

2. Kit Components and Storage Conditions

2-1. Kit Components (48 Preps)

Following contents are included.

The kit contains total RNA extraction reagents and consumables for 48 samples.

<input type="checkbox"/> Lysis Buffer LRV	42.5 ml
<input type="checkbox"/> Reagent Strip	48
<input type="checkbox"/> 1 ml Long Tips	48
<input type="checkbox"/> Waste Tubes	48

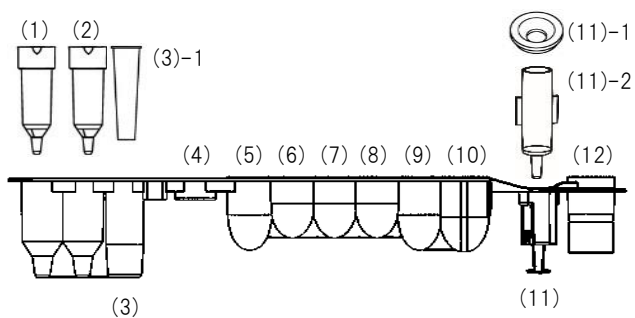
2-2. Storage Conditions

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

2-3. Reagent Strip Components

Each reagent strip contains the following contents.

Well Number	Composition/Reagent/Purpose	Classification	Standard
(1)	Short Tip	Molded Item	2/Strip
(2)	Short Tip	Molded Item	
(3)	1 ml Long Tip Insert Position	Molded Item	1/Strip
(3)-1	Tip Pack		1/Strip
(4)	Sample tube Set Position		1/Strip
(5)	EtOH (>99%) Ethanol)	Reagent	185 µl/Well
(6)	SRV (Solubilization Buffer)	Reagent	185 µl/Well
(7)	-	-	-
(8)	WRV (Wash Buffer)	Reagent	750 µl/Well
(9)	WRV (Wash Buffer)	Reagent	750 µl/Well
(10)	WRV (Wash Buffer)	Reagent	750 µl/Well
(11)	Cartridge Set Position		1/Strip
(11)-1	Pressure Adapter	Molded Item	1/Strip
(11)-2	Cartridge	Molded Item	1/Strip
(12)	ERV (Elution Buffer)	Reagent	250 µl/Well



3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

* Prepare if necessary

- Carrier RNA

Recommended products

- Poly(A) (Sigma-Aldrich : P4903-25MG)
- Poly(A) (Roche : 10108626001)
- Carrier RNA (Thermo Fisher Scientific : 4382878)
- Carrier RNA (Poly rA) (Qiagen : 1068337)

[2] Equipment

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

Recommended products: 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 strip and the heater part of the equipment beforehand.

- 1. 5 ml or 2 ml microtubes for elution of RNA

Recommended products: 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

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/>)

◆ SRV (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.

◆ WRV (Wash Buffer)

- Do not drink or ingest. Avoid contact with eyes.
- Caution should be exercised with respect to fire because it contains 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.

◆ LRV (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.

◆ CRV (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.

◆ EtOH (Ethanol)

- Do not drink or ingest. Avoid contact with eyes.
- Caution should be exercised with respect 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.

- ◆ Use or storage of LRV at high temperature should be avoided.

- ◆ Any solution and waste fluid containing LRV 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°C – 28°C).

5. Precautions

◆ Handling of Starting Material

- When using potentially infectious samples, take appropriate biohazard measures.
- When handling potentially infectious samples, wear appropriate protective products (protective clothing, protective cap, lab coat, gloves(double), mask, etc.).
- For samples to be extracted for the first time with this kit, start the extraction from pharyngeal swab and conduct the preliminary experiment.
- Do not overload the cartridge, as this will significantly reduce RNA yield and quality. In the worst case, the cartridge may clog.
- The amount of sample that can be processed may vary depending on the condition of the samples.

◆ Use of Reagent

- If the precipitates are formed in LRV, dissolve them by incubation at 37°C and return 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 50 µl of CRV. The volume of CRV may be changed, however, the efficiency of elution may also change depending on the sample condition.
- 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 (Lysate 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.

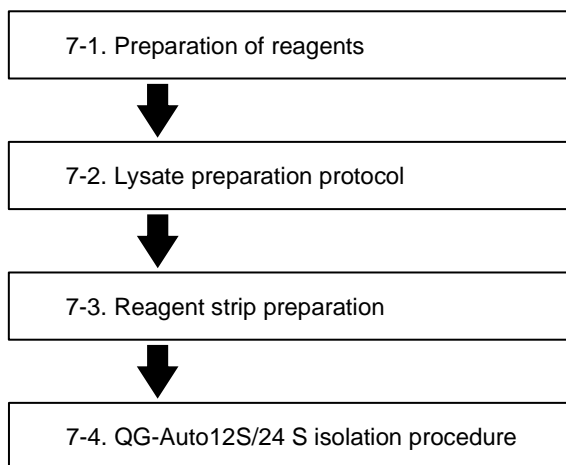
- Glassware or metal tools should be oven baked at 200 °C for 16 hours or more.

6. Quality Control

- We ensure that all lots meet our quality control standards.
- The yield and quality of Total RNA are confirmed by the amplification efficiency of RT-PCR.

7. Protocol

[Overview Flow Chart]



7-1. Preparations of Reagents

◆ LRV

Mix thoroughly before use.

If the precipitates are formed, dissolve them by incubation at 37°C, and return to room temperature before use.

◆ Carrier RNA solution

Prepare RNA solution following the below chart.

◇ Recommended Carrier RNA solution and adding volume

Products	Manufacturer	Cat.No.	Adding Volume
Poly(A)	Sigma-Aldrich	P9403-25MG	20 µg /sample
Poly(A)	Roche	10108626001	
Carrier RNA (Poly rA)	Qiagen	1068337	
Carrier RNA	Thermo Fisher Scientific	4382878	

The way for adjustment

Adjust to add 20µg per sample.

Adjust the concentration of the Carrier RNA solution so that the solution volume is within a maximum of 20µL.

7-2. Lysate Preparation Protocol

This kit is basically designed for the extraction of viral RNA from samples such as pharyngeal swab, culture cell supernatant liquid and serum. Since the lysate preparation protocol differs depending on the type of samples used, please check the type of samples and select the appropriate protocol.

[Important Notice before starting the extraction]

- Reagent should be brought to room temperature before using.
- The volume of reagent should be strictly in accordance with the volume specified in each lysate preparation flow.
- It is recommended to replace the pipette tip every time to prevent cross contamination.
- All process should be done in the room temperature(15~28°C).
- Do not leave any time in the middle of the extraction, and the operation should be done quickly.
- Never mix solutions or waste liquids containing LRV with bleach.
- When using samples that may be infectious, do the process in the BSL2+ laboratory or safety cabinet in the BSL2 laboratory.
- When do the pretreatment of potentially infectious samples, please do the process in the safety cabinet in the BSL2 laboratory.
- If you use samples that may be infectious and dispose of them after use, they fall under the category of infectious industrial waste, so please dispose of them appropriately.
- Wear appropriate protective equipment to reduce the risk of injury or infection from chemicals.
- Wear gloves when using the reagent strips and each tube to avoid contamination with nuclease.
- Please read the instruction manual of the QG-Auto 12S/24S.

7-2-1. Protocol for RNA Extraction from Culture Cell Supernatant Fluid

Be sure to follow the sequence of steps <1> through <4>.

If the order is changed, the desired RNA yield may not be obtained.

- <1> Add 200 μ L of LRV in the 2.0ml microtube.
- <2> Add designated volume of adjusted Carrier RNA solution.
Add 20 μ g of Carrier RNA solution per sample.
Adjust the concentration of the Carrier RNA solution so that the solution volume is within a maximum of 20 μ L.
For example, if you are adding 1 mg/mL Carrier RNA solution, add 20 μ L of Carrier RNA solution.
- <3> Add 200 μ l of cultured cell supernatant fluid.
- <4> Vortex at maximum speed for 30 seconds. Spin down for a few seconds to collect any liquid adhering to the lid or wall of the microtubes.

7-2-2. Protocol for RNA Extraction from Swab (nasopharynx, nasal cavity, or pharynx)

- <1> Add 200 μ L of LRV in the 2.0ml microtube.
- <2> Add designated volume of adjusted Carrier RNA solution.
Add 20 μ g of Carrier RNA solution per sample.
Adjust the concentration of the Carrier RNA solution so that the solution volume is within a maximum of 20 μ L.
For example, if you are adding 1 mg/mL Carrier RNA solution, add 20 μ L of Carrier RNA solution.
- <3> Add 200 μ l of swab (nasopharynx, nasal cavity, or pharynx).
- <4> Vortex at maximum speed for 30 seconds. Spin down for a few seconds to collect any liquid adhering to the lid or wall of the microtubes.
- <5> Incubate for 10 minutes in the room temperature.
When using potentially infectious samples, incubate them in a safety cabinet in the BLS2+ laboratory.

7-3. 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 2ml microtubes containing the lysate and 1 ml Long Tips in the specified position (p.5 (4)).
Make sure that the contents of the reagent strip are set in the correct position.

7-4. 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> Select [CUSTOMIZED PROTOCOL] from home screen, and select [RNA VIRUS].
- <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.

8. Trouble-shooting

When troubles occur, please refer below measures.

(1) Low yield or no RNA obtained

Causes	Measures
Inappropriate storage conditions for the tissue sample.	Yield of RNA varies depending upon the type, amount, storage period, and storage conditions of each sample. Do not use any sample once thawed, if it is not used immediately.
Inappropriate amount of sample to be treated.	Perform extraction within an appropriate amount of sample. Ex. Designated amount for Nasopharyngeal swab is 200 μ l.
Insufficient agitation after adding LRV.	After adding LRV, mix thoroughly. If nasopharyngeal swab or serum is used as a sample, incubate for 10 minutes after mixing.
Not added specified amount of Carrier RNA solution.	Add the carrier RNA in the lysate so that the amount of carrier RNA is 20 μ g. The amount of carrier RNA solution to be added should be 20 μ l or less.
Carrier RNA solution has deteriorated.	Do not refreeze and use Carrier RNA solution once it has been thawed.

(2) Clogging of Cartridge occurs

Causes	Measures
Not using appropriate protocol for the sample	Select the correct protocol for the type and volume of samples.
Inappropriate amount of processed samples.	Perform extraction within an appropriate amount of sample. Ex. Designated amount for Nasopharyngeal swab is 200 μ l.
Insufficient agitation after adding LRV.	After adding LRV, mix thoroughly. If nasopharyngeal swab or serum is used as a sample, incubate for 10 minutes after mixing.
When centrifuging the cell culture solution and separating the supernatant, the residue (cultured cells) was taken along with the supernatant.	Repeat the centrifugation or extend the centrifugation time to ensure removal of the residue (cultured cells).

(3) Subsequent experiments such as RT-PCR etc. do not proceed well

Causes	Measures
Not added specified amount of Carrier RNA solution.	Add the carrier RNA in the lysate so that the amount of carrier RNA is 20 μ g. If the amount of Carrier RNA added is small, the RNA collection rate may decrease.
Carrier RNA solution has deteriorated.	Do not refreeze and use Carrier RNA solution once it has been thawed.

9. 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 RNA Tissue Kit	48 preps	AS-RT
QuickGene-AutoS RNA Cultured Cell Kit	48 preps	AS-RC
QuickGene-AutoS DNA FFPE Kit	48 preps	AS-DF

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