KURABO

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HANDBOOK

QuickGene-AutoS RNA Cultured Cell Kit (AS-RC)

For extraction of total RNA from cultured cell

Ver.1.1

Contents

1. Introduction 4
2. Kit Components and Storage Conditions 4
2-1. Kit Components (48 Preps) 4
2-2. Strage Conditions 4
2-3. Reagent strip components 5
3. Other Required Materials, Not Supplied in This Kit 5
4. Safety Warnings..6
5. Precautions 7
6. Quality Control 8
7. Product Description 8
8. Protocol 9
8-1. Preparation of Reagents 9
8-2. Lysate Preparation Protocol 10
8-3. Reagent strip preparation 10
8-4. QG-Auto12S/QG-Auto24S 10
9. Troubleshooting 13
10. Ordering Information 15

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-Auto12S or QG-Auto24S, high quality and high yield total RNA can be extracted and also purified from cultured cell.
- RNA from cultured cell samples can be simultaneously extracted in following time.
  QG-Auto12S：about 20 min for 12 sets
  QG-Auto24S：about 20 min for 24 sets
- The purified, high quality total RNA is suitable for PCR, restriction enzyme digestion, NGS analysis and other applications.

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

2. Kit Content and Storage Conditions

2-1. Kit Components (48 Preps)

☐	Lysis Buffer LRC	37.5 ml
☐	Reagent strip	48 pcs
☐	1 ml Long Tips	48 pcs
☐	Waste Tubes	48 pcs

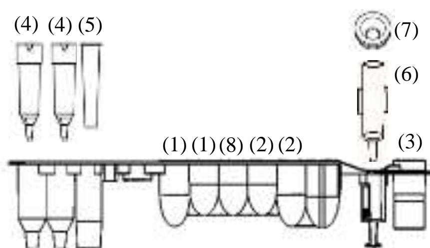
2-2. Storage Conditions

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

2-3. Reagent strip components

☐	(>99%) Ethanol	EtOH	100 µl	180ul	(1)
☐	Wash Buffer	WRC	500 µl	2 positions	(2)
☐	Elution Buffer	CRC	250 µl		(3)
☐	Short Tip		2		(4)
☐	Tip pack		1		(5)
☐	Cartridge		1		(6)
☐	Pressure Adapter		1		(7)
☐	DNase solution※				(8)

※ Used in case of DNase treatment



3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

- 2-mercaptoethanol (2-ME) (used in LRC)

※ Reagents to be prepared as necessary

- DNase

  <Recommended goods>

  ・ RQ1 RNase-Free DNase (Promega:Cat. No. M6101)

  ・ Deoxyribonuclease(RT Grade) (Nippon Gene: Cat. No. 313-03161)

  ・ DNaseI, RNase-Free (Life Technologies:Cat. No. AM2222)

  ・ RNase-Free DNase Set (QIAGEN:Cat. No. 79254)

[2] Equipment

- QuickGene-Auto12S/QuickGene-Auto24S
- Micropipette and tips

  Recommendation product : BM EQUIPMENT Cat. BM4020
  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 equipment heater part beforehand.

- 1.5 ml or 2 ml microtubes for elution of RNA

Recommendation product： BM EQUIPMENT Cat. BM4015,
SARSTEDT Cat.72.706.700

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

- Tube stand
- Tube mixer (capable of agitation about 2,500 rpm)
- Simple desk top centrifuge (capable of centrifuging about 5,000×g)

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/)

◆ LRC (Lysis Buffer)

- Drinking may be harmful.
- Do not drink or ingest. Avoid contact with eyes.
- Handle in well-ventilated area.
- Wear appropriate protective gloves and goggles when handling this chemical.
- Do not use or store this chemical in a fire or hot place.
- Keep the container tightly closed.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ WRC (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.

◆ CRC (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.
- Flammable liquid is included. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.
- Caution should be exercised with respected to fire because it is a highly flammable liquid.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

- ◆ Use or storage of Reagent strips at the specified temperature (15ºC – 28ºC).
- ◆ LRC should not be used or stored in warm areas.
- ◆ Never mix the LRC-containing solution or waste liquid 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.

5. Precautions

◆ Handling of Starting Material

- This kit is for total RNA isolation from up to $1x10^6$ cells. If the cell number is too much, cell lysis may not be sufficient, membrane may clog or yield may be reduced.
- If membrane clog, reduce the sample amount.
- Yield is up to sample status. If the sample volume is large, cell lysis may not be sufficient or yield may be reduced.

◆ Use of Reagent

- LRC may precipitate during storage. If any deposits occur, melt at 37°C and allow to warm to room temperature before use.

◆ Procedure of Extraction

- All operations should be performed at room temperature (15°C to 28°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Do not take time during separation and perform the procedure quickly.
- This kit assumes dissolution with 50 µl of CRC. The amount of CRC may be changed, but the dissolution efficiency may 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 (Lysed cells 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.
- 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.

<Preventing RNase Contamination>

- Wear appropriate gloves when handling RNA or separating reagents to prevent contamination of the RNase.
- It is recommended to use RNase free or sterile plastic products.
- If glass or metal products are used, dry-heat sterilize at 200°C for at least 16 hours before use.

◆ Other Precautions

- Non-denaturing gel electrophoresis of the collected total RNA may result in banding tailings on the polymer side, but there are no problems with RNA quality.

6. Quality control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD. the performance of QuickGene-AutoS RNA Cultured Cell Kit (AS-RC) is evaluated routinely on a lot-to-lot uniformity.
- Yield and quality of extracted plasmid DNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

7. Product Description

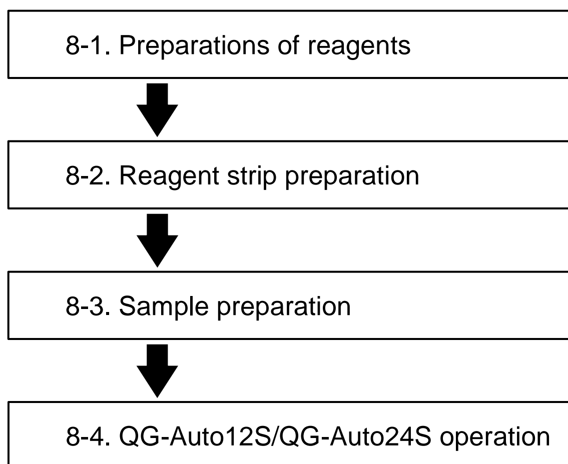
This kit corresponds to the isolation and purification of total RNA from cultured cells (up to 1×10^6). Examples of total RNA yield and purities per 1 × 10 6 of cultured cells are shown in Table 1.

Table1. Examples of total RNA yields from cultured cell (HL60, 1×10^6 cells) with DNase treatment

Sample	Yield (μg)	A260/280
HL60(1×10^6 cells)	9.8	2.29

8. Protocol

〔Overview Flow Chart〕



8-1. Preparation of Reagent

◆ LRC

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

◆ 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	Manufacture	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)

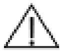
2U / µl DNase I	20 µl
10×Reaction Buffer	4 µl
Nuclease Free Water	16 µl

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



Note: Be sure to follow the sequence of steps <1> to <3>.
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> Count the number of cells exactly.

If the number of cells is too many, it may cause significant decrease in the yield or precision of extraction, or sometimes clogging. When clogging occurs, reduce the number of cells, and then try again.

<1a> To lyse pelleted cells :

・ To make pellet from adherent cells :
Please collect, and count the cells by the trypsin processing. Centrifuge cells for 5 min at 300 × g, and aspirate supernatant.

・ To make pellet from cells grown in suspension :
Pellet cells for 5 min at 300 × g in a tube. Carefully remove all supernatant, and wash the pelleted cells with PBS. Centrifuge it at 300 × g for 5 min again. Carefully remove supernatant.
Pelleted cells can be stored at –70°C if it is frozen with liquid nitrogen. Please make sure to count the number of cells before freezing.

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

<2> Add LRC to lyse cells.

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

<2a> To lyse pelleted cells :
Loosen cells by fl icking the microtube and then add 520 µl of LRC.
When a frozen pellet is used, add about 20 µl of PBS to the pelleted cells, resuspend cells by flicking, and then add LRC.

<2b> To lyse cells directly in cultured dish (On-dish lysis) :
Add 520 µl of LRC to a fl ask or a dish, mix cells and LRC well with the aid of a cell scraper or the like, and transfer the whole lysed cells to a microtube.

<3> Mix by tube mixer for 1 min at the maximum speed. Then, flash spin down for several seconds to remove drops from the inside of the lid.

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.

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 cultured cell”.
<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 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 Cultured Cell Kit (AS-RC).

(1) Low yield or no RNA obtained

Causes	Measure
Insufficient removal of culture medium from a flask or dish	If the culture medium remains, the concentration of LRC may be diluted and yield may be reduced. Remove as much culture solution as possible from the flask or dish.
Inadequate cell number	Count the number of cells and separate them within the appropriate cell count range.
2-ME not added to LRC	Dispense the required volume of LRC prior to use and add 10 µl of 2-mercaptoethanol (2-ME) per 1 ml of LRC.
Insufficient dispersion of cell pellets	Tap the pellet sufficiently and completely unwind. Especially in the case of frozen pellets, add 20 µl of PBS after cell thawing, tap thoroughly, and completely loosen the pellet.
Precipitate is present in the LRC	Before use, confirm that the LRC does not contain any deposits. If any deposits are observed, warm at 37°C and allow the precipitate to warm to room temperature after dissolution before use.
Insufficient homogenization after addition of LRC	Vortex at maximum speed for 1 minute.
Without added DNase reaction buffer (for DNase treatment)	Check the addition of a predetermined amount of DNase reaction buffer during the preparation of the DNase solution

(2) The purity of RNA is low.

Causes	Measure
The number of cell is too large.	Reduce the number of cells.

(3) Clogging of Cartridge (CA) occurs

Causes	Measure
The number of cell is too large.	Reduce the number of cells.
Insufficient dispersion of cell pellets	Tap the pellet sufficiently and completely unwind. Especially in the case of frozen pellets, after cell lysis, add 20 µl of PBS and tap it sufficiently to completely loosen the pellet.
Vortex after addition of LRC is insufficient	Vortex at maximum speed for 1 minute.
Vortex is Insufficient	Vortex after addition of LRC is recommended for 1 minute in this kit, but longer vortexing may improve clogging.

(4) RNA degradation

Causes	Measure
2-ME not added to LRC	Dispense the required volume of LRC prior to use and add 10 µl of 2-mercaptoethanol (2-ME) per 1 ml of LRC.
Contamination of the RNase	RNase may be contaminated during operation and storage. Be careful not to contaminate the RNase.
RNase contamination in DNase (In case of adding DNase treatment)	Use the recommended RNase-Free DNase. Contact your manufacturer for more information about the DNase.
RNA was warmed	RNA may break down when warmed. Handle RNA on ice as much as possible during use.

(5) Subsequent experiments such as PCR etc. do not proceed well

Causes	Measure
Inappropriate amount of RNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Contamination of genomic DNA	Perform DNase treatment according to the DNase treatment methods. If DNA degradation is incomplete, see (6).

(6) Incomplete degradation of DNA (In case of adding DNase treatment)

Causes	Measure
No specified quantity of DNase solution added to the specified area	Add the DNase solution to the specified area of the reagent strip (see p. 5, Reagent strip components). See Reagents p. 9-10 Prepare the recommended DNase solutions.
Insufficient DNase activity	Use the recommended DNase activity.

(7) Precipitates occurred in the reagent.

Causes	Measure
Stored at low temperature	Store this kits at room temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 37°C. Cool down it to room temperature before use.

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 RNA Tissue Kit	48 preps	AS-RT
QuickGene-AutoS RNA Cultured Cell Kit	48 preps	AS-RC

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