KURABO

HANDBOOK

QuickGene-AutoS RNA Blood Kit (AS-RB)

For extraction of total RNA from leukocyte

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 post-hemolytic blood cells (up to 1.5 x 10^7 cells derived from healthy adult blood.)
- RNA from leukocyte can be simultaneously extracted in following time.
  - QG-Auto12S: about 45 minutes for 12 sets of leukocyte samples
  - QG-Auto24S: about 45 minutes for 24 sets of leukocyte 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 LRB	37.5 ml
☐	Reagent strips	48
☐	1 ml Long Tips	48
☐	Waste Tubes	48

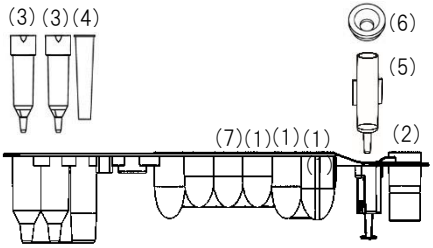
2-2. Storage Conditions

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

2-3. Reagent strip components

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

*Used with DNase treatment



3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

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

* Prepare if necessary

- DNase [For optional process. Recommended products are listed as below.]
  - RQ1 RNase-Free DNase (Promega: Cat. No. M6101)
  - Deoxyribonuclease (RT Grade) (NIPPON GENE: Cat. No. 313-03161)
  - DNase I, RNase-Free (Thermo Fisher Scientific: Cat. No. AM2222)
  - RNase-Free DNase Set (QIAGEN: Cat. No. 79254)

[2] Equipments

- QuickGene-Auto12S/QuickGene-Auto24S
- Micropipettes and tips (RNase-free)

- 2 ml microtubes for samples
  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 (maximum speed at 2,500 rpm or more)
- Benchtop microcentrifuge (maximum speed at 5,000 xg or more)

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/)

◆ LRB(Lysis Buffer)

- Harmful if ingested.
- Do not drink or ingest. Avoid contact with eyes.
- It should be handled at a well-ventilated place. Wear a laboratory coat, gloves and safety goggles during experiments.
- If contact with eyes, skin or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Use or storage of this buffer close to fire or at high temperature should be avoided.
- Keep the bottle tightly closed.

◆ WRB (Wash Buffer)

- Highly flammable liquid. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.
- 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.

◆ CRB(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 Reagent strips at the specified temperature (15ºC – 28ºC).

◆ Any solution and waste fluid containing LRB 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.

5. Precautions

◆ Handling of Starting Material

- Do not use frozen blood samples.
- The yield varies depending upon sample conditions (health condition which blood derived from).
- In case clogging occurs, try by reducing the number of leukocytes.

◆ Use of Reagent

- If the precipitates are formed in LRB, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.
- Use or storage of LRB at high temperature should be avoided.
- Any solution and waste fluid containing LRB should not be mixed with bleach.

◆ 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.
- During the procedure, work quickly without interruption.
- This kit assumes dissolution with 50 µl of CRB. The amount of CRB 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.
  - 1 ml Long tips and 2 ml microtubes (Whole blood and EDB included) are set in the Reagent strip.
  - Reagent strips are set correctly in the Reagent holder.
  - 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 QuickGene-Auto12S / QuickGene-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 Blood Kit (AS-RB) 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

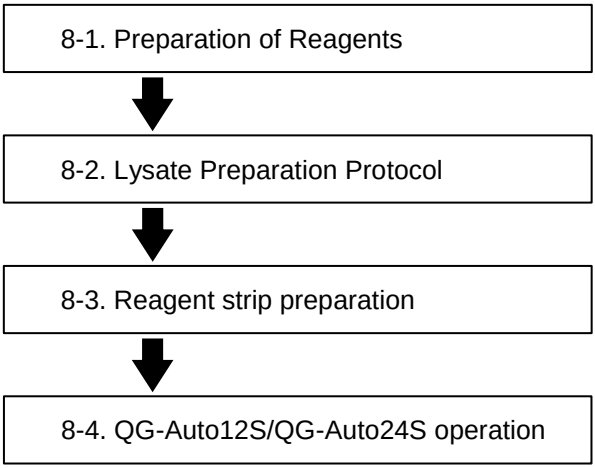
This kit corresponds to the extraction of total RNA from leucocytes (1.5×10^7 cells or less) after hemolysis. About 4,000-7,000 leucocytes are included in each µl of blood from healthy adults. Thus about 2 ml of the blood including 7,000 leukocytes/µl provides the maximum number of leucocytes (1.5×10^7 cells) suitable for this kit. Table 1 shows the example of yield and purity the total RNA extracted from different numbers of blood cells (in the case with DNase treatment).

Table 1. Yield and purity of total RNA

Sample	Yield (µg)	A260/280
1.5×10^7	5.1	2.30

8. Protocol

[Overview Flow Chart]



8-1. Preparations of Reagents

◆ LRB

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 LRB before each use. Add 10 µl 2-ME per 1 ml of LRB. Use 520 µl of LRB per 1 sample. 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	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

Note: Be sure to follow the sequence of steps <1> to <3>.
If the order is changed, the desired yield may not be obtained.

- Count the number of leukocytes, confirm that it is under 1.5×10^7 cells before use.
  About 4,000-7,000 leucocytes are included in each µl of blood from healthy adults.
  For example, 2 ml of blood with containing 7,000 leukocytes/µl is proper for 1.5×10^7 cells per sample.
- 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> Use a pellet of leukocytes in a 1.5 ml microtube, after erythrocytes lysis.
In case of using excessive amounts leukocytes, extraction would end with the following results : clogging, low yield, and low purity. Please make sure the number of leukocytes is under 1.5×10^7 cells. When clogging occurs, reduce the number of cells, and then try again.
Add 2-ME to LRB before use (p.9).
<1a> Pelleted leukocytes (-1.5×10^7) in 1.5 ml microtube, after erythrocytes lysis :
Loosen the pelleted cells thoroughly by flicking the tubes. Add 520 µl of LRB (2-ME added). Mix LRB and cells thoroughly by pipetting.
<1b> Pelleted leukocytes (-1.5×10^7) in except 1.5 ml microtube, after erythrocytes lysis :
Loosen the pelleted cells thoroughly by flicking the tubes. Add 520 µl of LRB (2-ME

added). Mix LRB and cells thoroughly by pipetting, and transfer into a 1.5ml microtube.

<2> Vortex at the maximum speed for 30 sec. Flash spin down for several seconds to remove drops from the inside of the lid.
Make sure to vortex at the maximum speed for 30 sec.

<3> Add 250 µl of >99% ethanol. Vortex at the maximum speed for 5 min. Flash spin down for several seconds to remove drops from the inside of the lid.
As in step <2>, make sure to vortex at the maximum speed for 5 min.
Optional procedure : For the best result, it is recommended to add a ball (zirconia 5 mmφ) during homogenization. When using a ball, use 2 ml microtube.

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 Blood cell”.
<4> Choose 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 Blood Kit (AS-RB).

(1) Low yield or no RNA obtained

Cause	Measure
2-ME not added to LRB	Dispense the required volume of LRB prior to use and add 10 µl of 2-mercaptoethanol (2-ME) per 1 ml of LRB.
Insufficient lysis of leukocyte	Check that there is no precipitate in LRB. In case a precipitate is formed, dissolve the precipitate by incubation at 37°C. Use it after cooling back to room temperature.
Insufficient vortexing after addition of LRB (2-ME added)	Vortex thoroughly (See section 8-2 p.11).
No addition of the prescribed volume of DNase Reaction Buffer (When using a DNase)	When using a DNase, check that the prescribed volume of DNase Reaction Buffer has been added.
RNA degradation	Refer to (3) “RNA degradation”.
Temperature of operation is high	Take all of operation at room temperature (15-28°C).

(2) Clogging of Cartridge occurs:

Cause	Action
Use of too much amount of leukocytes	Reduce the amount of leukocytes.
Insufficient vortexing after addition of LRB or ethanol	After LRB or ethanol addition, perform vortexing completely for 30 sec or 5 min, respectively, according to section 8-2 (p.11). Pipette several times when transferring lysate to the Cartridge. Optionally put a ball (zirconia 5 mmφ) in a 2 ml tube before vortexing for 5 min after ethanol addition.
Insufficient vortexing after addition of LRB	Vortex thoroughly at the maximum speed.
Vortexing is insufficient	Vortex after addition of LRB is recommended for 1 minute in this kit, but longer vortexing may improve clogging.

(3) RNA degradation

Cause	Measure
No addition of 2-ME to LRB	Dispense a required volume of LRB before use, and add 10 µl of 2-mercaptoethanol (2-ME) to each ml of LRB.
RNase contamination	RNase contamination may occur during preparation and storage. Extreme caution is required to avoid RNase contamination.
RNase contamination in DNase (when using a DNase)	Use any one of the recommended RNase-free DNase. For the details, inquire to each manufacture.

Heating of RNA	RNA may be degraded when heated. Store RNA samples on ice during experiments.

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

Cause	Measure
Inappropriate amount of RNA is used	Determine the RNA 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 (5).
RNA degradation	Refer to (3) “RNA degradation”.

(5) Incomplete degradation of DNA (when using a DNase):

Cause	Measure
Required volume of DNase is not added at the specified position	Add the DNase solution to the specified area of the reagent strip (see p.5, Reagent strip components). See p.9-10 Preparation of Reagents to prepare the recommended DNase solutions.
Insufficient DNase activity	Use a recommended DNase activity.

(6) A precipitate is formed in reagents :

Cause	Measure
Stored at low temperature	Store buffers at the prescribed temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 37°C. Use it after cooling back to room temperature.

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

Appendix 1 Erythrocyte Lysis

We introduce a hemolysis method carrying out as an example in our company.

Hemolytic agent (HB)

NH_4Cl	150mM
$NaHCO_3$	10mM
EDTA(pH8.0)	0.1mM

1. Mix 1 volume of human whole blood with 5 volumes of HB in an appropriately sized tube (not provided).
   For example, add 5 ml of HB to 1 ml of whole blood and mix well.
   Notice: Use an appropriate amount of whole blood. Up to 1.5 × 107 leukocytes of healthy blood (typically 4,000-7,000 leukocytes per µl) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used.

2. Incubate for 10-15 min on ice. Mix by inverting tubes or vortexing briefly 2 times during incubation.
   The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 min.

3. Centrifuge at 2,000 × g for 2 min at 4°C, and completely remove and discard supernatant.
   Leukocytes will form a pellet after centrifugation. Remove supernatant carefully, do not disturb pellet.

4. Add HB to the cell pellet (use 2 volumes of HB per volume of whole blood used in step 1. Resuspend cells by vortexing well.
   For example, add 2 ml of HB per 1 ml of whole blood used in step 1.
   Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in this wash step. If erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5-10 min on ice after addition of HB at this step.

5. Centrifuge at 2,000 xg for 2 min at 4°C, and completely remove and discard supernatant.

According to protocol, advance to the extraction step.
*After erythrocyte lysis, all of the extraction step should be performed as quickly as possible.

* Trademark and exclusion item
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KURABO INDUSTRIES LTD.

Bio-Medical Department, Advanced Technology Division

14-30, Shimokida-Cho, Neyagawa,
Osaka 572-0823, Japan
TEL +81-72-820-3079 FAX +81-72-820-3095
URL; http://www.kurabo.co.jp/bio/English/

AS-RB_HB-E_V10