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<b>HANDBOOK</b>
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# **QuickGene-AutoS RNA FFPE Kit (AS-RF)**

**For extraction of total RNA from FFPE**

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## Warning

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

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# 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 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, total RNA can be extracted and also purified from FFPE samples.
- DNA from tissue lysate samples can be simultaneously extracted in following time.
  - QG-Auto12S: about 115 min for 12 sets of FFPE samples
  - QG-Auto24S: about 115 min for 24 sets of FFPE samples
- The purified, total RNA is suitable for PCR, restriction enzyme digestion, NGS analysis and other applications.

**Please be sure to read the Operation Manual of QuickGene Auto-12S / QuickGene Auto-24S carefully before using this kit.**

## 2. Kit Components and Storage Conditions

### 2-1 Kit Components (48 Preps)

<input type="checkbox"/> Deparaffinization Buffer DRF	1 vial
<input type="checkbox"/> Proteinase K ERF	1 vial
<input type="checkbox"/> Reagent strips	48
<input type="checkbox"/> 1 ml Long Tips	48
<input type="checkbox"/> Waste Tubes	48

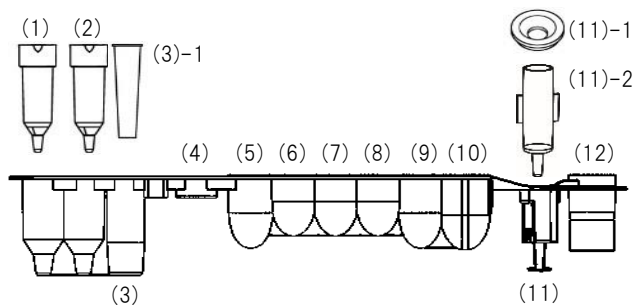
### 2-2 Storage Conditions

All reagents are stable at room temperature (15-28°C) until expiring date indicated at outer box. We suggest keeping ERF at 2-8°C to prolong its life.

## 2-3. Reagent strip components

No	Materials	quantity
(1)	Short Tip	2
(2)	Short Tip	
(3)	1 ml Long Tip ( set position)	1
(3)-1	Tip Pack	1
(4)	Sample tube (set position)	1
(5)	MRF (Tissue Lysis Buffer)	240 $\mu$ l
(6)	LRF (Lysis Buffer)	190 $\mu$ l
(7)	DNase solution*	
(8)	EtOH ( ( >99% ) Ethanol)	400 $\mu$ l
(9)	WRF (Wash Buffer)	750 $\mu$ l
(10)	WRF (Wash Buffer)	1500 $\mu$ l
(11)	Cartridge (set position)	1
(11)-1	Pressure Adapter	1
(11)-2	Cartridge	1
(12)	CRF 'Elution Buffer)	500 $\mu$ l

\*Used with DNase treatment



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## 3. Other Required Materials, Not Supplied in This Kit

### [1] Reagents\*

\* 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] Equipment

- QuickGene-Auto12S or QuickGene-Auto24S
- Micropipettes 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 strip and equipment heater part beforehand.

- 1.5 ml or 2 ml microtubes for elution of RNA

Recommendation product: BM EQUIPMENT Cat. 4015, Cat.4020

SARSTEDT Cat.72.706.700

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

- Tube stand
- Microcentrifuge (c.a. 12,000 rpm)
- Vortex Mixer

## 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/>)

### ◆ DRF (Deparaffinization 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.

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- Wear a laboratory coat, gloves and safety goggles during experiments.
- ◆ ERF (Proteinase K)
- 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.
- ◆ MRF (Tissue Lysis 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.
  - Wear a laboratory coat, gloves and safety goggles during experiments.
- ◆ LRF (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.
- ◆ WRF (Wash Buffer)
- Include flammable liquids, so be careful with the fire
  - 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.
- ◆ CRF (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)
- 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.
- ◆ Use or storage of Reagent strips at the specified temperature (15°C – 28°C).
- ◆ Any solution and waste fluid containing LRF 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.

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## 5. Precautions

### ◆ Handling of Starting Material

- QuickGene-AutoS RNA FFPE S Kit (AS-RF) basically corresponds to total RNA extraction from 1 to 10 FFPE sections with a thickness of 5 to 10  $\mu\text{m}$ .

**Table 1 :** Amount of FFPE section can be processed

Thickness	number of sheets
5 $\mu\text{m}$	1 to 10
10 $\mu\text{m}$	1 to 5

※You can use surface area up to 120 mm.

- The maximum amount of FFPE sections may be decreased from the respective values shown in Table 1, depending upon the site, condition of a tissue sample.
- If you use QuickGene-AutoS RNA FFPE Kit (AS-RF) for the first time, start the separation from 1 to 10 5  $\mu\text{m}$  FFPE sections. Performing a preliminary test is recommended.
- Do not overload the Cartridge (CA), as this will significantly reduce total RNA yield and quality. In the worst case, the Cartridge may clog.
- If clogging occurs, clean the reagent holder and collection holder.

### ◆ Use of Reagent

- If the precipitates are formed in MRF during storage, dissolve them fully by incubating at 55°C. Cool down it to room temperature before use.
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### ◆ Procedure of Extraction

- Before starting operation, please make sure the following things:
  - Add 40  $\mu\text{L}$  of ERF at the location of reagent strip (5).
  - 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 (containing FFPE sections) 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°C - 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 QuickGene-Auto12S / QuickGene -Auto24S for details.



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## 6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene-AutoS RNA FFPE Kit (AS-RF) is evaluated routinely on a lot-to-lot uniformity.

## 7. Product Description

QuickGene-AutoS RNA FFPE kit S (AS-RF) corresponds to the extraction of total RNA from FFPE sections, basically FFPE with a thickness of 5 to 10 $\mu$ m.

Table 2 shows examples of total RNA yield and purity when this kit is used for extraction from normal mouse tissue (A260/280).

**Table 2 :** Examples of Yields and purities of total RNA..

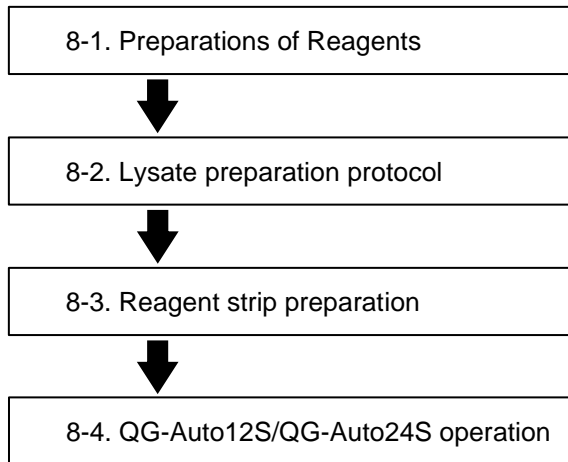
Sample	number of intercept	$\mu$ g	A260/280
Mouse fetal	10 $\mu$ m $\times$ 3	29.1	2.0

- Yields and purity may vary depending on the sample species.
- The default volume of CRF is 100  $\mu$ l. The minimum elution volume is 50  $\mu$ l, however the efficiency of elution may decrease when the volume collected is very small.

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## 8. Protocol

### [Overview Flow Chart]



### 8-1. Preparations of Reagents

#### ◆ DRF

Mix thoroughly 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/40 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 / $\mu$ l DNase I	20 $\mu$ l
10 $\times$ Reaction Buffer	4 $\mu$ l
Nuclease Free Water	16 $\mu$ l

### Preparation 2)

2U / $\mu$ l DNase I	20 $\mu$ l
10 $\times$ Reaction Buffer	4 $\mu$ l
Nuclease Free Water	16 $\mu$ l

### Preparation 3)

2.7Kunitz units / $\mu$ l DNase I <sup>*2</sup>	1.25 $\mu$ l
Buffer RDD	35 $\mu$ l
Nuclease Free Water	3.75 $\mu$ l

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

## 8-2. Lysate preparation protocol

### Notices

Follow the protocol of <1> to <5> exactly.

In case the procedure is changed, the yield of RNA may not be obtained.

- Wear a suitable laboratory coat, disposable gloves and safety goggles during the experiments.
- To avoid contamination of nuclease, wear disposable gloves during preparation of Reagent strips and microtubes.
- Refer to the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for details.

<1> Prepare an FFPE embedded sample.

Use the specified amount (basically 1 to 5  $\mu$ m) of the FFPE sections that have been cut out.

If the amount of sections is too large, clogging may occur and the yield and purity may decrease significantly. If it is clogged, consider reducing the section volume

<2> Place the FFPE sections in a 2 ml microtube.

<3> Add 5 drops of DRF.

DDF should be dropped onto the sample as much as possible so that it does not hit the wall of the tube.

<4> Vortex at maximum speed for 15 seconds.

<5> Centrifuge at room temperature for 3 minutes at 12,000 rpm.

Collect the sections at the bottom of the tube. If a section remains on the top of the tube, stir again and centrifuge.

## 8-3. Reagent strip preparation

- To avoid contamination of nuclease, wear disposable gloves during preparation of Reagent strips and microtubes.
- Refer to the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for details.

<1> Prepare the Collection holder and Reagent holder on the workbench.

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- <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 the 2 ml microtube containing FFPE sections and 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 Customized protocol screen, select the "RNA FFPE".
  - <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 4°C or -20°C.
    - In case of storing total RNA for a long time, it is recommended to preserve them at -80°C.

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## 9. Troubleshooting

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

### (1) Low yield or no RNA obtained

Cause	Action
Incomplete tissue dissolution	Add ERF at position (5) on the reagent strip. Refer to "2-3. Reagent Strip Contents" when adding.
Use of too much amount of the FFPE sample	Reduce the sample amount to the specified amount (see Table 1, p.7).

### (2) Clogging of Cartridge (CA) occurs

Cause	Action
Use of too much amount of the FFPE sample	Reduce the sample amount to the specified amount (see Table 1, p.7).
Incomplete tissue dissolution	Add ERF at position (5) on the reagent strip. Refer to "2-3. Reagent Strip Contents" when adding.

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

Cause	Action
Inappropriate amount of RNA is used	Check the density with a Qubit® fluorometer.
Low purity of RNA	Refer to (1) "Low yield or no RNA obtained urity of RNA is low".

### (4) A precipitate is formed in reagents

Cause	Action
Stored at low temperature	Store this kit at room temperature (15-28°C).

## 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
QuickGene-AutoS DNA FFPE Kit	48 preps	AS-DF
QuickGene-AutoS RNA Virus Kit	48 preps	AS-RV

\* Trademark and exclusion item

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