With QuickGene-Mini480

KURABO

Ver.16-08 E

Isolation of total RNA Quick Guide

Tissue RNA

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QuickGene RNA tissue kit S Ⅱ (RT-S Ⅱ)

In this Quick Guide, the protocol for isolation of total RNA from animal tissue is a digest from the Handbook of QuickGene RNA tissue kit S Ⅱ (RT-S2) and the Operation Manual of QuickGene-Mini480. * **Before using, please read the Operation Manual.**

Wear protective gloves and safety goggles during the experiments.

step1 Preparations

In order to isolate the target total RNA, please prepare the following items.

1 Preparations

QuickGene-Mini480

RNA tissue kit S Ⅱ (RT-S2)

High grade ethanol (>99%)

2-Mercaptoethanol (2-ME)

DNase (refer to step1-2)

Microcentrifuge

Safety goggles

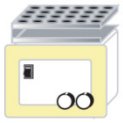
Protective gloves

Microtubes (1.5 ml)

Micropipettes (P-200, P-1000 or other types)

Tube mixer

or



Homogenizer

or

or

Use matching tubes and beads with different homogenizer.

2 Preparation of Reagents

◆ Lysis Buffer (LRT)

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubating at 37℃.

2-Mercaptoethanol (2-ME) must be added to LRT before each use. Add 10 µl of 2-ME per 1 ml of LRT.

Use 500 µl of LRT (with 2-ME) per 1 sample (1 Cartridge).

※ All steps of the protocol should be performed at room temperature. Any solution and waste should not be mixed with bleach.

◆ Solubilization Buffer (SRT)

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubating at 37℃.

◆ Wash Buffer (WRT)

Add 280 ml of ethanol (>99%) into the bottle of WRT and mix with inversion the bottle gently at the begging of use.

Close the cap firmly to prevent volatilizing and store it at room temperature.

◆ Elution Buffer (CRT)

Use CRT for elution of RNA.

◆ DNase [For optional process. Recommended products are listed as below.]

In case of performing the DNase treatment, prepare the DNase solution according to the following tables.
Prepare the DNase reaction solution before using. (Value in the table is for 1 cartridge.)

Product name	Manufacturer	Cat.No.	Preparation	Final Conc.
RQ1 RNase-Free DNase	Promega	M6101	1	20 U/40 µl
Deoxyribonuclease (RT Grade)	Nippon Gene	313-03161		
DNase I, RNase-Free	Thermo Fisher Scientific	2222	2	40 U/40 µl
RNase-Free DNase Set※1	QIAGEN	79254	3	3.4 Kunitz units/40 µl

Preparation,1

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

Preparation,2

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

Preparation,3

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

※1: Add 550 µl of RNase-free water to the bottle of 1,500Kunitz units DNase1. This is the DNase stock solution. (Refer to the User's Manual with DNase.)

※2: In case of using QIAGEN protocol to prepare DNase solution, the DNase might be hyperactivity. The above condition is recommended for preparation of DNase solution.

3 Amount of Starting Material (tissue sample)

QuickGene RNA tissue kit S Ⅱ (RT-S2) corresponds to total RNA isolation from 5~30 mg of mammalian tissue sample.

Check the amount of sample in following table, or refer to the handbook of RT-S2 kit.

1) Examples for yields of total RNA obtained from normal tissues of Balb/c mouse (female, 7 weeks old)
(With DNase treatment; homogenize with a TOMY Micro Smash MS-100 or QIAGEN Tissue Lyser.)

Tissue	Maximum sample weight [mg]	(example) total RNA yield※2 [µg]
Liver	30	100~120
Brain		15~20
Lung		20~25
Kidney		50~60
Spleen		40~50
Thymus		40~60
Heart		15~20

2) Treatment amout of sample, depend on the type of homogenizer (Balb/c mouse (female 7 weeks old))

Tissue	Ball mill [mg]	Rotor-Stator [mg]	Pestle [mg]
Liver	30	15	15
Brain	40	40	20
Lung	30	15	15
Kidney	30	5	×
Spleen	30	20	10
Thymus	30※3	5	5
Heart	30※3	5	×

× : unusable

※1: Use higher efficient of homogenizing condition for Thymus and Heart than other tissue.
※2: The yield of total RNA is depending on the tissue condition or part.
※3: Extend the homogenizing time with TOMY Micro Smash MS-100 if the cartridge glogs at worst.

In case of using Rotor-Stator homogenizer or pestle homogenizer, the yield of RNA might be 30~50% lower than the case of using ball mill homogenizer.

Preliminary experiment with using 10 mg tissue for the following cases.

1) The sample which is first isolated by using QuickGene series.
2) The sample which is first isolated.
3) The sample which is not in the table above.

When tissue sample is not used immediately, freeze it in liquid nitrogen and save it at -80℃.

Continue to Step2

step2 protocol

In order to gain the target yield of total RNA, please follow the protocol below.

Use fresh tissue or frozen one (-80℃) from animals.

The volume of the eluate from each cartridge is 100 µl.
The volume of CRT can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

If tissue volume is not appropriate, the yield and the purity of RNA might decrease or the cartridge (CA) might clog.
Reduce the volume of tissue when the cartridge clog.

1 Measurement of tissue

1) Mix LRT and 2-ME in accordance with the number of samples . (Add 10 µl of 2-ME per1 ml of LRT)

2) Cut tissue into1.5~2 mm square and measure the weight.

3) Transfer required amount of tissue into the homogenizer-compliant tubes.
(In case of using ball mill homogenizer, put 5 mmΦ zirconia beads into tubes before transferring tissue.)

Keep frozen condition of tissue during measurement by using dry ice or liquid nitrogen to avoid RNA degradation.

2 Add LRT (with 2-ME), then homogenize

1) Ball mill homogenizer
Add 500 µl of LRT (with 2-ME) and homogenize to make uniform lysate according to the instruction manual of the homogenizer. Decide the homogenizing condition according to the following table.

Homogenizing condition for 5~15 mg tissue

Tissue	TOMY MicroSmash MS-100	QIAGEN TissueLyser
Liver	3,800 rpm, 120 sec	30 Hz, 5 min ×2 times
Brain		
Lung		
Kidney	3,800 rpm, 300 sec	
Spleen		
Thymus		
Heart		

Homogenizing condition for 15~30 mg tissue

Tissue	TOMY MicroSmash MS-100	QIAGEN TissueLyser
Liver	3,800 rpm, 300 sec	30 Hz, 5 min ×2 times
Brain		
Lung		
Kidney		
Spleen		
Thymus	3,800 rpm, 240 sec ×2 times	
Heart	3,800 rpm, 300 sec ×3 times	

※ For Thymus : TOMY MicroSmash MS-100R (with cooler) is better than MS-100 to get higher yield.

2) Rotor-Stator homogenizer
Add 500 µl of LRT (with 2-ME) , then homogenize at 20,000 rpm for 30 sec ×2 times.

Use 2 ml microtubes for 7 mmΦ probes.
Use larger tubes for over 10mmΦ probes.

Extend the homogenizing time if tissue pieces remain.

3) Pestle homogenizer for microtubes
Add 200 µl of LRT (with 2-ME) and homogenize immediately for over 1 min by the pestle homogenizer .
Homogenize completely by pressing the pestle against the bottom of a tube and a little get it off sometimes.
After the homogenization, add 300 µl of LRT (with 2-ME) and voltex for 15 sec.

※ Kidney and heart are not suitable for this homogenization.

3 Isolation of supernatant and tissue pieces.

1) Centrifuge at ≧17,000x g for 3 min at room temperature.

If tissue pieces are transferred altogether, the cartridge might clog.
When tissue pieces are not isolated well or excess bubbles are formed, increase the speed or extend the time of centrifugation.

2) Be careful not to draw tissue pieces at the bottom of the tube.

- 5～15 mg tissue : transfer 350 µl of supernatant to a new 1.5 ml tube
- 15～30 mg tissue : transfer 385 µl of supernatant to a new 1.5 ml tube

4 Add SRT, then voltex for 15 sec at the maximum speed.

Add 175 µl of SRT, then voltex for 15 sec at the maximum speed .
After the above step, flash spin down for several seconds to remove drops from the inside of the lid.

When homogenate is lost, change ratio of SRT and ethanol as follows.

- 5～15 mg tissue : homogenate:SRT:ethanol = 2:1:1
- 15～30 mg tissue : homogenate:SRT:ethanol = 11:5:4

5 Add ethanol (>99%), then voltex for 1 min at the maximum speed.

1) - 5～15 mg tissue : Add 175 µl of ethanol (>99%)
   - 15～30 mg tissue : Add 140 µl of ethanol (>99%)

2) Voltex for 1 min at the maximum speed.

3) Then, flash spin down for several seconds to remove drops from the inside of the lid.

6 Complete the lysis

Perform the isolation operation within 30 min, after completing the lysis.

Continue to Step3

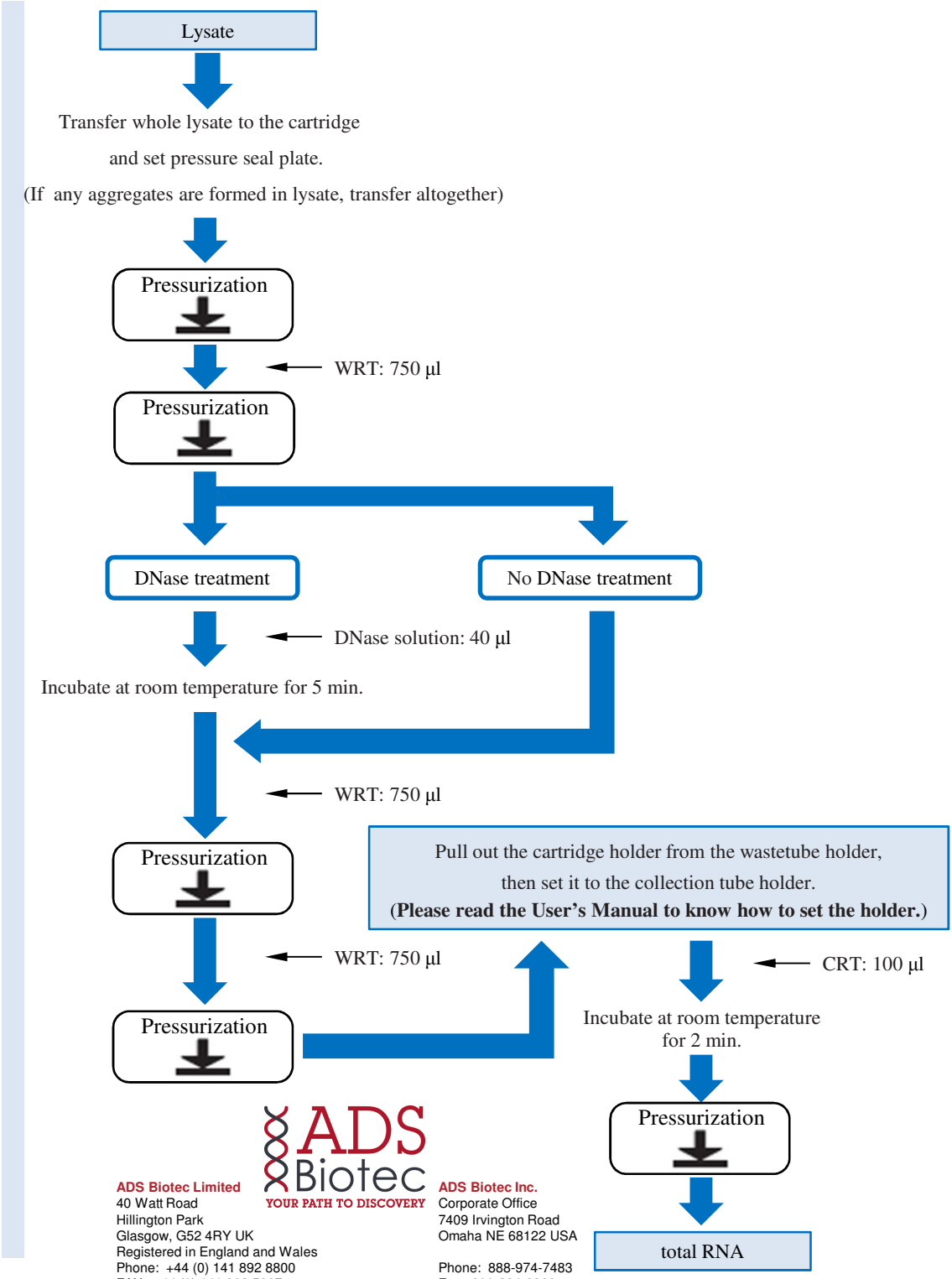
step3 Isolation protocol with QuickGene-Mini480

Use QuickGene-Mini480 to isolate total RNA from tissue sample.

1 QuickGene-Mini480 Workflow

The Pressurization mark in the workflow indicates the following operations.

①Set holder into system. ※**Please read the User's Manual to know how to set the holder.**
②Rotate pressurizing switch toward the front side to start pressurizing.
③Make sure that there is no residual liquid in the cartridge and return the pressurizing switch to original position.
④Move the holder to pressurize the next row. Repeat ② and ③.
⑤Pull out holder from system.



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