



QuickGene Series Application Guide

Total RNA Isolation from Leukocyte

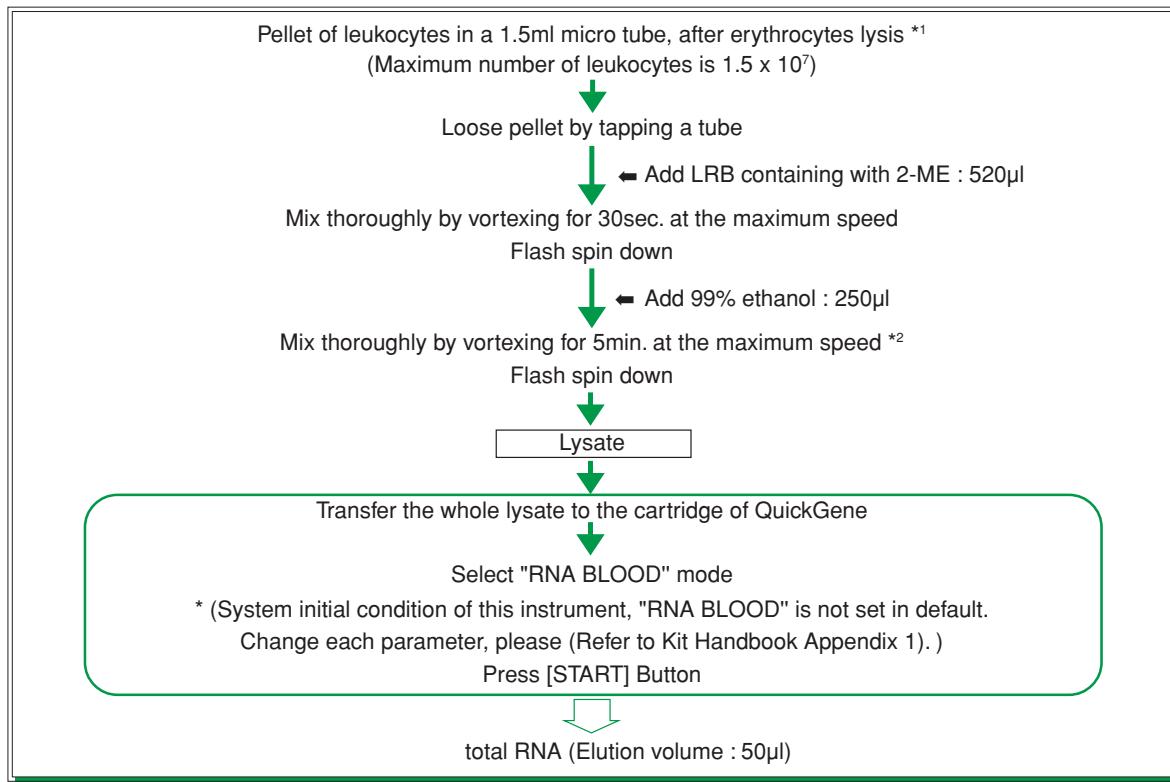
Kit : QuickGene RNA blood cell kit S
Model : QuickGene-810

Summary

- This is the kit for total RNA isolation from leukocyte prepared from fresh human blood.
- Enables high-purity total RNA isolation from 1.5×10^7 human leukocyte by simple pretreatment.
- After setting lysate prepared by pretreatment, isolation is completed for about 20min. for 8 samples at the same time.
- Isolated total RNA is available for RT-PCR and real time PCR.

Protocol 1

● Without DNase treatment

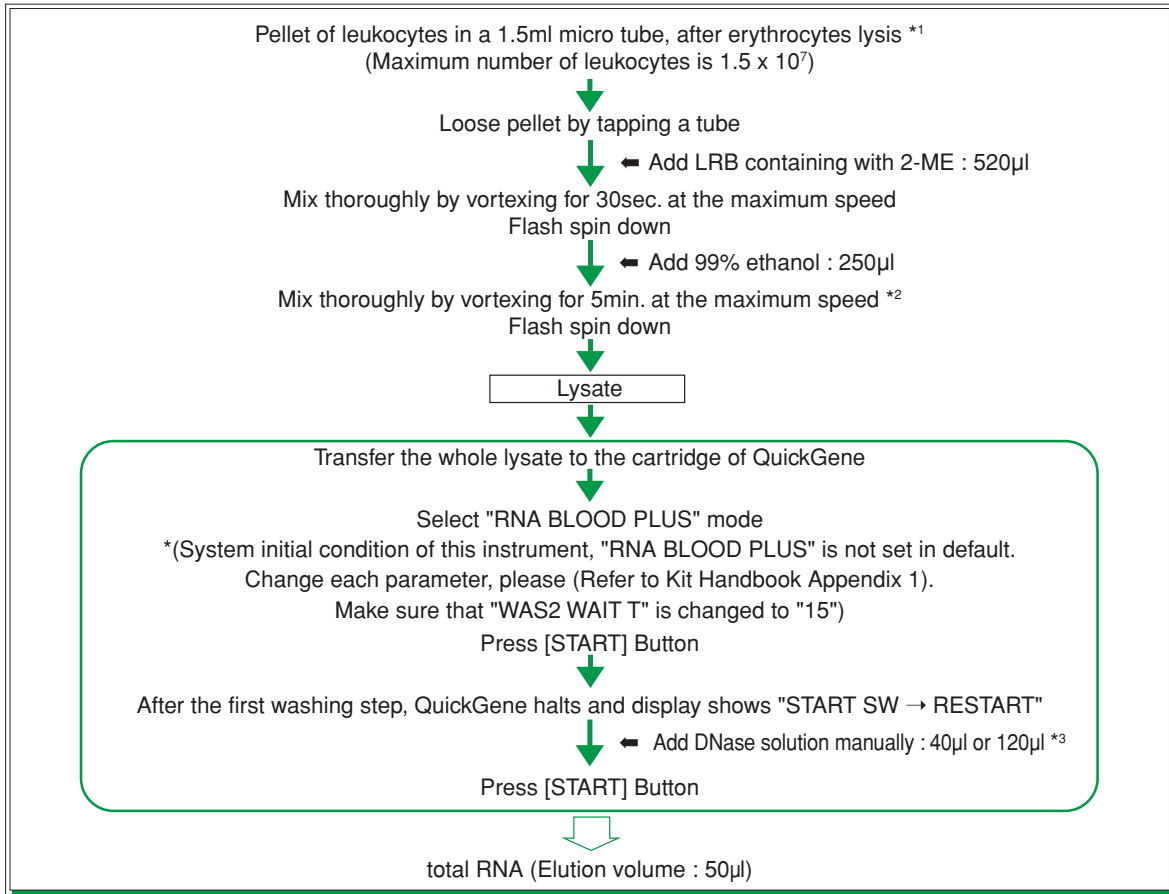


*1 : As to erythrocyte lysis method, please refer to p.4 "Erythrocyte lysis".

*2 : Putting a zirconia ball (5mm ϕ) into a tube is effective procedure for complete vortexing.
At the time, please use 2ml micro tube.

Protocol 2

● With DNase treatment



*1 : As to erythrocyte lysis method, please refer to p.4 "Erythrocyte lysis".

*2 : Putting a zirconia ball (5mm ϕ) into a tube is effective procedure for complete vortexing.
At the time, please use 2ml micro tube.

*3 : Please use the following recommended DNase.

Recommended DNase

- | | |
|---------------------------------|-----------------------------------|
| a) RQ1 RNase-Free DNase | (Promega : Cat. No. M6101) |
| b) DNase I, Amplification Grade | (Invitrogen : Cat. No. 18068-015) |
| c) RNase-Free DNase Set | (QIAGEN : Cat. No. 79254) |
| d) DNase I, Amplification Grade | (Sigma : Cat. No. AMP-D1) |

DNase reaction solution of a), b) : Add 40µl

1U/µl DNase I	: 20µl
10 x Reaction Buffer	: 4µl
RNase-free water	: 16µl

DNase reaction solution of c) : Add 40µl

2.7Kunitz units/µl DNase I	: 1.25µl
Buffer RDD	: 35µl
RNase-free water	: 3.75µl

DNase reaction solution of d) : Add 120µl

1U/µl DNase I	: 60µl
10 x Reaction Buffer	: 12µl
RNase-free water	: 48µl

Preparation of reagents

- Lysis Buffer (LRB)
Dispense the necessary volume of LRB, then add 10µl of 2-mercaptoethanol (2-ME) per 1ml of LRB prior to use.
- Wash Buffer (WRB)
Add 120ml of > 99% ethanol into the bottle and mix with inversion the bottle gently before using.

Example of total RNA isolation from Leucocyte

Total RNA was isolated from Leucocytes after erythrocyte lysis using QuickGene system (QuickGene-810 and QuickGene RNA blood cell kit S).

● The yield and purity of total RNA

	Number of leukocytes	QuickGene		Spin column method(A company) *1		Automatic magnetic bead method *2	
		RNA yield (μg)	A _{260/280}	RNA yield (μg)	A _{260/280}	RNA yield (μg)	A _{260/280}
With DNase treatment	2 x 10 ⁶	0.6	2.20	0.4	2.04	0.7	2.46
	1 x 10 ⁷	4.5	2.21	3.8	2.09	-	-
	1.5 x 10 ⁷	6.5	2.10	-	-	-	-
Without DNase treatment	1 x 10 ⁷	5.0	2.17	4.2	2.10	-	-

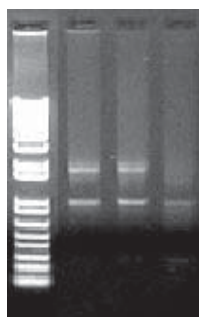
*1 : For spin column method, maximum number of leukocytes is 1 x 10⁷.

*2 : For automatic magnetic bead method, maximum number of leukocytes is 2 x 10⁶.

● Electrophoresis of total RNA

Number of leukocytes : 2 x 10⁶
DNase(+)

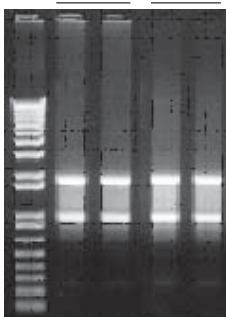
M 1 2 3



← 28S
← 18S

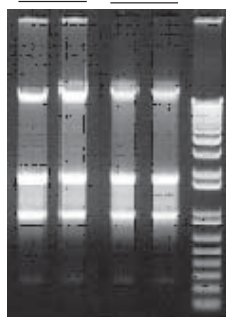
Number of leukocytes : 1 x 10⁷
DNase(+)

M 1 2



DNase(-)

1 2 M



Electrophoresis condition : 1% Agarose / 1 x TAE

M : Marker

(1Kb Plus DNA Ladder : Invitrogen)

1 : QuickGene

2 : Spin column method(A company)

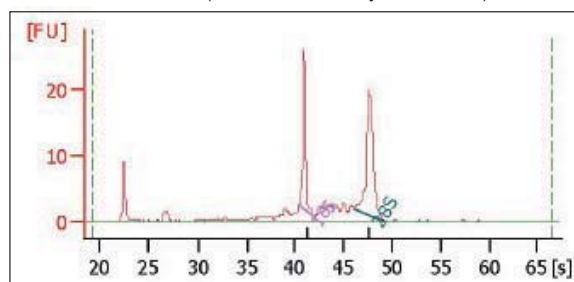
3 : Automatic magnetic bead method

For Automatic magnetic bead method, yield of isolated RNA was low, and 28S band was hardly detected. On the other hand, RNA was isolated in high yield for QuickGene.

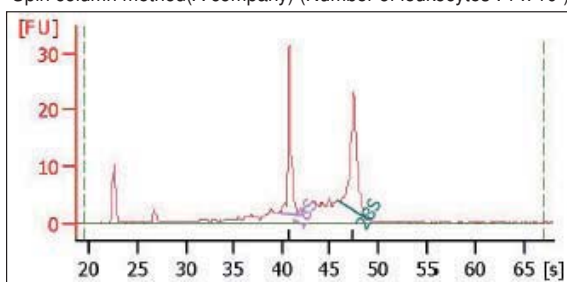
● The quality of total RNA (with DNase treatment)

Total RNA was isolated from leukocytes after erythrocyte lysis using QuickGene system, Spin column method(A company) and Automatic magnetic bead method. Then, it was analysed with Agilent 2100 Bioanalyser RNA 6000 Nano LabChip® kit.

QuickGene (Number of leukocytes : 1 x 10⁷)



Spin column method(A company) (Number of leukocytes : 1 x 10⁷)



	Number of leukocytes	QuickGene	Spin column method (A company)	Automatic magnetic bead method
RIN	2 x 10 ⁶	7.7	6.5	5.0
	1 x 10 ⁷	9.2	8.8	-
28S / 18S	2 x 10 ⁶	1.5	0.8	0.0
	1 x 10 ⁷	1.6	1.2	-

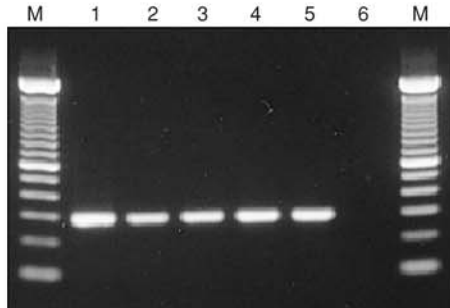
RIN (RNA integrity number : Agilent) :

an indicator of quality of RNA available for array and so on the best value : RIN=10.

By use of QuickGene system high-quality total RNA available for array analysis was obtained.

● RT-PCR

RT-PCR was performed on total RNA isolated from 5×10^5 leukocytes after erythrocyte lysis using QuickGene system and Spin column method(A company). After reverse transcription reaction (RT) using $5\mu\text{l}$ of isolated total RNA lysate and then dilution, PCR reaction was carried out with GAPDH primer.



M : Marker (100bp DNA Ladder : Invitrogen)
 1 : Positive control
 2,3 : QuickGene
 4,5 : Spin column method(A company)
 6 : Negative control

For RT-PCR performed on total RNA lysate isolated from 5×10^5 leukocytes after erythrocyte lysis, electrophoretic bands of the amplification products were detected.

● Real Time PCR

Number of copied GAPDH per $1\mu\text{g}$ of total RNA (For isolation from 1×10^7 leukocytes)

QuickGene	3.15×10^7
Spin column method(A company)	1.11×10^7

Used model : Real Time PCR system Roche LightCycler
 Used reagents : LightCycler FastStart DNA Master SYBR Green I
 LightCycler Human GAPDH Primer Set

Erythrocyte lysis

Mix 1 volume of whole human blood with 5 volumes of HB *1 in an appropriately sized tube (not provided).

* Use 40ml of HB *1 when you treat 10ml of blood in 50ml tube.

Incubate for 10 - 15min. on ice. Mix by inverting tubes or vortexing briefly 2 times during incubation.

Centrifuge at $2,000 \times g$ for 2min. at 4°C , and completely remove and discard supernatant.

Add HB *1 to the cell pellet (use 2 volumes of HB per volume of whole blood used in step 1) , Resuspend cells by vortexing well.

Centrifuge at $2,000 \times g$ for 2min. at 4°C , and completely remove and discard supernatant.

According to protocol 1, 2 on pages 1, 2, advance to the extraction step.

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

*1 : hemolytic agent (HB)

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

* As to the detail, refer to Kit Handbook.

* Besides this example, total RNA isolation from leukocytes separated by density-gradient centrifugation using Ficoll is also possible.

* Trade mark and exclusion item

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