11.	Total RNA Extraction from Blood of An	nimal



RA-a-1

Total RNA Extraction from Leukocyte

Protocol

Pellet of leukocytes in a 1.5 ml micro tube, after erythrocytes lysis (Maximum number of leukocytes is 1.5×10^7)

Loose pellet by tapping a tube

■ LRB containing with 2-ME *1 : 520 μl

Vortex (maximum speed) *1 : 30 sec

Flash spin down

→ >99% ethanol : 250 μl

Vortex (maximum speed): 5 min *2

Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)

total RNA (Elution volume : 50 μl) *1 Add 10 µl of 2-ME per 1 ml of LRB.

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

Results

The yield of total RNA / Protein contamination: A260/280

	Number of leukocytes	QuickGene		Spin column method (company A) *1		Automatic magnetic bead method *2	
	(cells)	(µg)	A260/280	(µg)	A260/280	(µg)	A260/280
	2 x 10 ⁶	0.6	2.20	0.4	2.04	0.7	2.46
With DNase treatment	1 x 10 ⁷	4.5	2.21	3.8	2.09	1	-
	1.5 x 10 ⁷	6.5	2.10	-	-	-	-
Without DNase treatment	1.0 x 10 ⁷	5.0	2.17	4.2	2.10	-	-

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

Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.

The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).



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

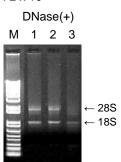


Electrophoresis of total RNA

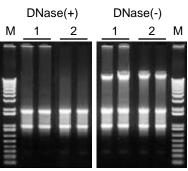
Number of leukocytes

Number of leukocytes

: 2 x 10⁶



: 1 x 10⁷



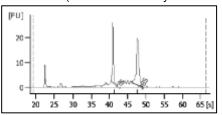
Electrophoresis condition: 1% Agarose / 1 x TAE M: Marker (1Kb Plus DNA Ladder: Invitrogen)

1: QuickGene

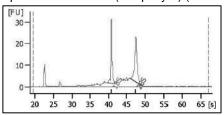
2 : Spin column method (A company) 3: Automatic magnetic bead method

The quality of total RNA (with DNase treatment)

QuickGene (Number of leukocytes: 1 x 10⁷)



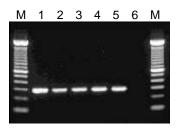
Spin column method (company A) (Number of leukocytes: 1 x 10⁷)



	Number of leukocytes	QuickGene	Spin column method (company A) *1	Automatic magnetic bead method *2
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
	1.0 x 10 ⁷	1.6	1.2	-

Other

RT-PCR



M: Marker (100bp DNA Ladder: Invitrogen)

1: Positive control 2,3: QuickGene

4,5 : Spin column method (A company)

6: Negative control

Real Time PCR

Number of copied *GAPDH* per 1µg of total RNA (For isolation from 1 x 10⁷ leukocytes)

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

Used model Real Time PCR system Roche LightCycler Used reagents

LightCycler FastStart DNA Master SYBR Green I

LightCycler Human GAPDH Primer Set

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data. The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).





