11. Total RNA Extraction from Blood of Animal

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 x 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
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	1.0 x 10^7	5.0	2.17	4.2	2.10	-	-

*1 : For spin column method, maximum number of leukocytes is 1 x 10^7.
*2 : For automatic magnetic bead method, maximum number of leukocytes is 2 x 10^6.

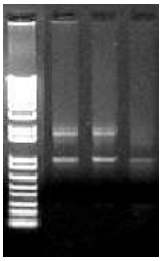
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Electrophoresis of total RNA

Number of leukocytes : 2 x 10^6

DNase(+)

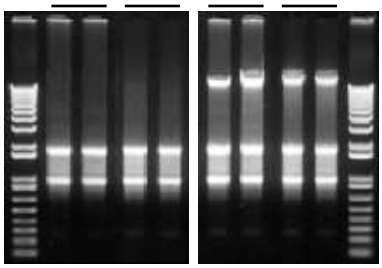
M 1 2 3



Number of leukocytes : 1 x 10^7

DNase(+) DNase(-)

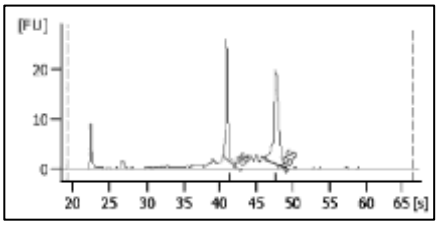
M 1 2 1 2 M



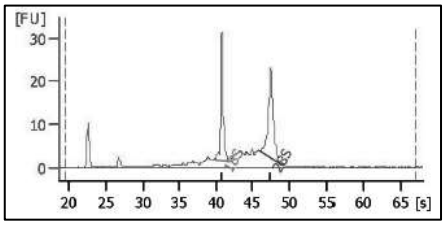
Electrophoresis condition : 1% Agarose / 1 x TAE
M : Marker (1Kb Plus DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
3 : Automatic magnetic bead method

The quality of total RNA (with DNase treatment)

QuickGene (Number of leukocytes : 1 x 10^7)



Spin column method (company A) (Number of leukocytes : 1 x 10^7)

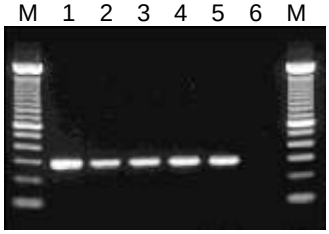


	Number of leukocytes	QuickGene	Spin column method (company A) *1	Automatic magnetic bead method *2
RIN	2 x 10^6	7.7	6.5	5.0
	1 x 10^7	9.2	8.8	-
28S / 18S	2 x 10^6	1.5	0.8	0.0
	1.0 x 10^7	1.6	1.2	-

Other

- RT-PCR

M 1 2 3 4 5 6 M



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 10^7 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

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