

## Chapter 3-XI-i

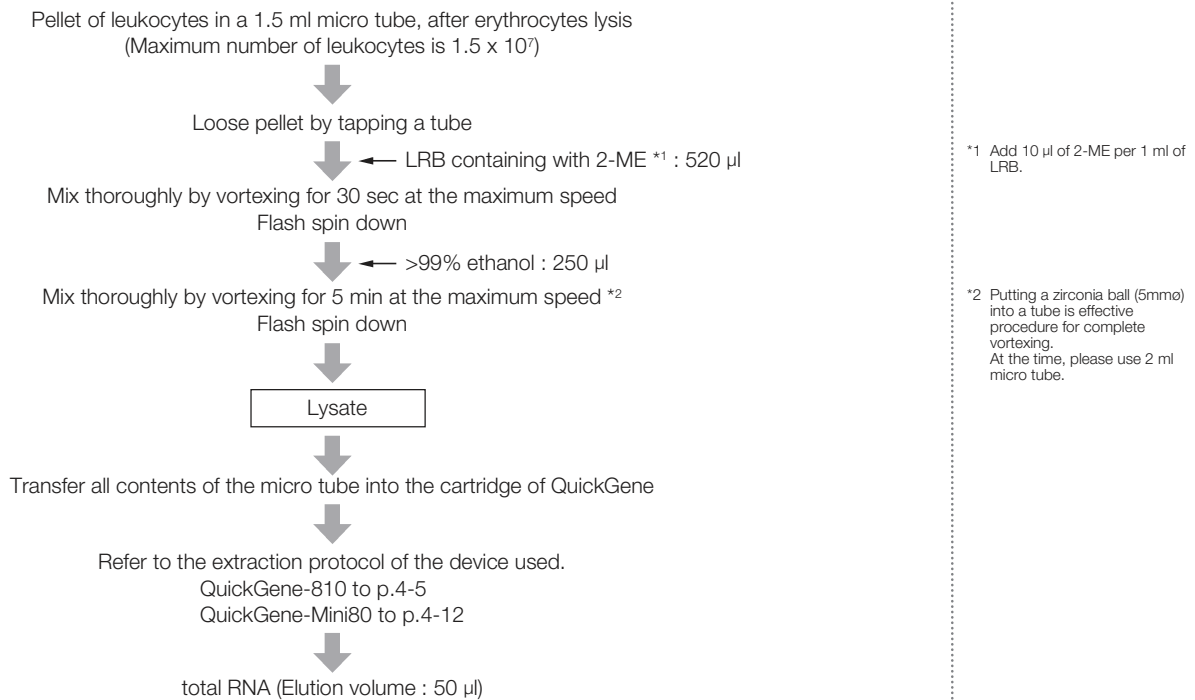
### Total RNA Extraction from Blood of Animal

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**RA-a-1**

# Total RNA Extraction from Leukocyte

## Protocol



## Results

### Electropherogram

No Data

### The yield of total RNA

	Number of leukocytes	QuickGene	Spin column method (A company) *1	Automatic magnetic bead method *2
With DNase treatment	2 x 10 <sup>6</sup>	0.6	0.4	0.7
	1 x 10 <sup>7</sup>	4.5	3.8	-
	1.5 x 10 <sup>7</sup>	6.5	-	-
Without DNase treatment	1.0 x 10 <sup>7</sup>	5.0	4.2	-

\*1 : For spin column method, maximum number of leukocytes is 1 x 10<sup>7</sup>.

\*2 : For automatic magnetic bead method, maximum number of leukocytes is 2 x 10<sup>6</sup>.

### Protein contamination : A260/280

	Number of leukocytes	QuickGene	Spin column method (A company) *1	Automatic magnetic bead method *2
With DNase treatment	2 x 10 <sup>6</sup>	2.20	2.04	2.46
	1 x 10 <sup>7</sup>	2.21	2.09	-
	1.5 x 10 <sup>7</sup>	2.10	-	-
Without DNase treatment	1.0 x 10 <sup>7</sup>	2.17	2.10	-

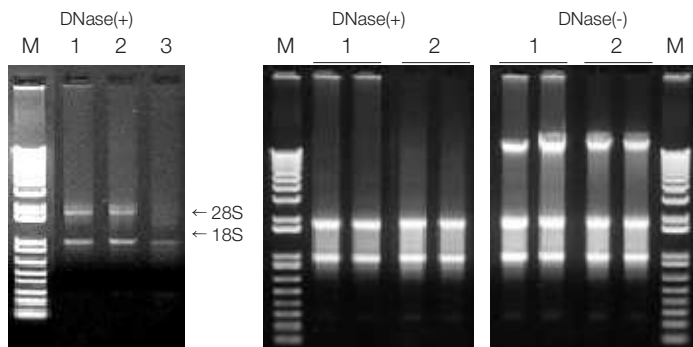
\*1 : For spin column method, maximum number of leukocytes is 1 x 10<sup>7</sup>.

\*2 : For automatic magnetic bead method, maximum number of leukocytes is 2 x 10<sup>6</sup>.

### Electrophoresis of total RNA

Number of leukocytes :  $2 \times 10^6$

Number of leukocytes :  $1 \times 10^7$



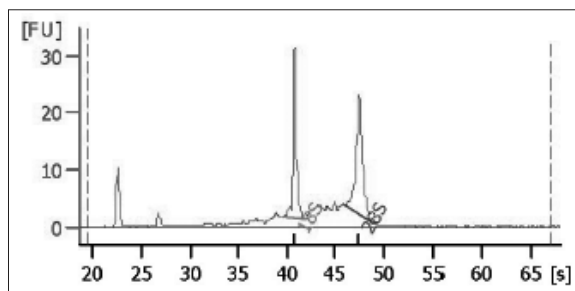
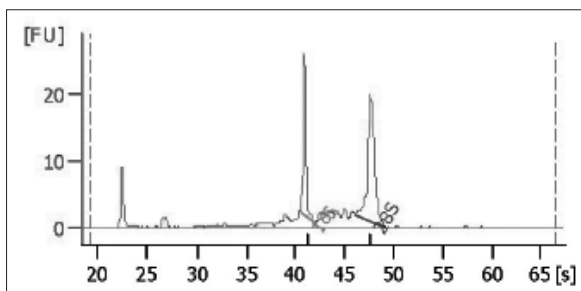
Electrophoresis condition : 1% Agarose / 1 x TAE

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 :  $1 \times 10^7$ )

Spin column method (A company) (Number of leukocytes :  $1 \times 10^7$ )

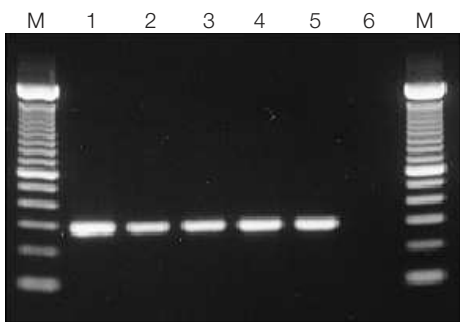


	Number of leukocytes	QuickGene	Spin column method (A company)	Automatic magnetic bead method
RIN	$2 \times 10^6$	7.7	6.5	5.0
	$1 \times 10^7$	9.2	8.8	-
28S / 18S	$2 \times 10^6$	1.5	0.8	0.0
	$1 \times 10^7$	1.6	1.2	-

RIN (RNA integrity number : Agilent) : an indicator of quality of RNA available for array and so on the best value : RIN=10.

### 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  $1 \times 10^7$  leukocytes)

QuickGene	$3.15 \times 10^7$
Spin column method (A company)	$1.11 \times 10^7$

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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