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 1.5×10^7)

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

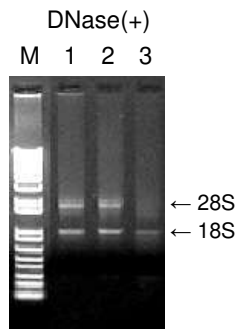|	Number of leukocytes	QuickGene		Spin column method (company A) [*1]		Automatic magnetic bead method [*2]	
	(cells)	(μg)	A260/280	(μg)	A260/280	(μg)	A260/280
With DNase treatment	2×10^6	0.6	2.20	0.4	2.04	0.7	2.46
	1×10^7	4.5	2.21	3.8	2.09	-	-
	1.5×10^7	6.5	2.10	-	-	-	-
Without DNase treatment	1.0×10^7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

*1 : For spin column method, maximum number of leukocytes is 1×10^7.

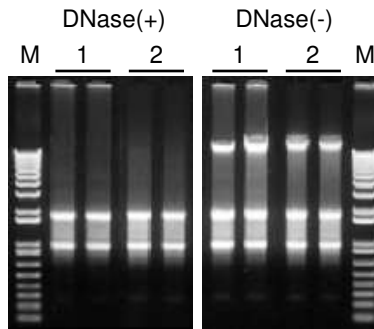*2 : For automatic magnetic bead method, maximum number of leukocytes is 2×10^6.

KIKURABO

Electrophoresis of total RNA

Number of leukocytes
: 2×10^6

Number of leukocytes
: 1×10^7

DNase(+)

M 1 2 3

DNase(+)

M 1 2

DNase(-)

1 2 M
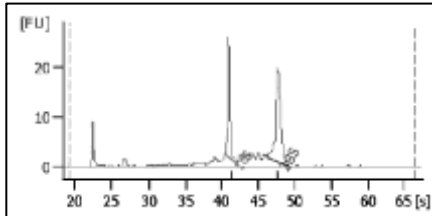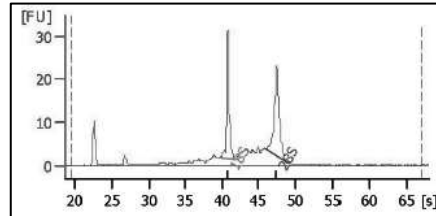
← 28S
← 18S

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 : 1×10^7)

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

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

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

Common protocol is usable for the following

No Data

KURABO