

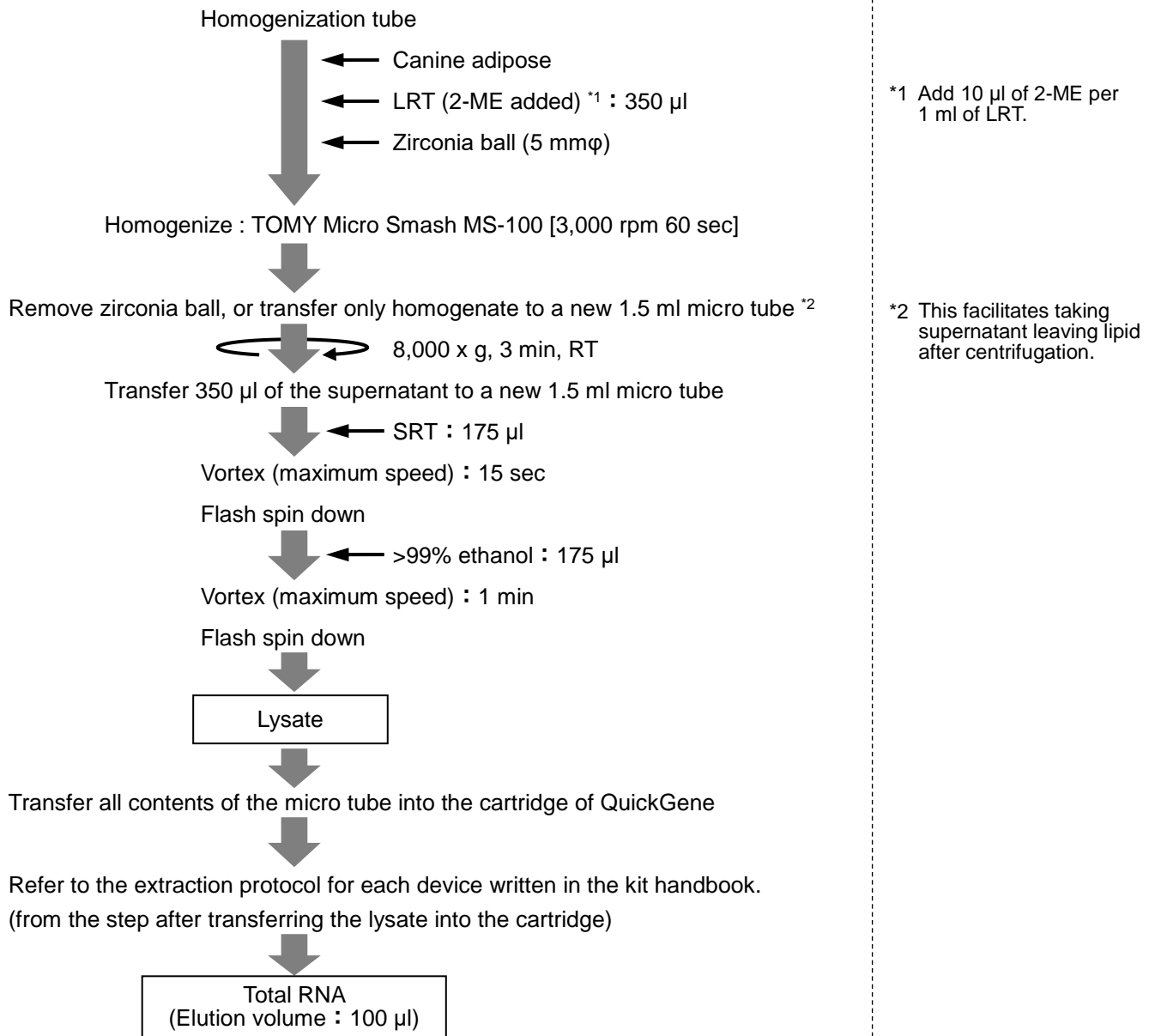


12. Total RNA Extraction from Tissue of Animal

RA-b-1

Total RNA Extraction from Adipose Tissue of Canine

Protocol



Results

Total RNA was extracted from canine or feline adipose tissue.

The yield of total RNA

Amounts of tissue	QuickGene (µg)	Competitor A kit (µg)
30 mg	0.5	0.8
100 mg	2.3	-
200 mg	4.6	4.2
400 mg	28.0	-

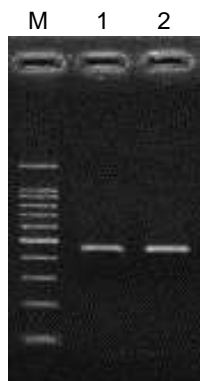
Protein contamination : A260/280

Amounts of tissue	QuickGene	Competitor A kit
30 mg	1.88	1.58
100 mg	2.12	-
200 mg	2.16	2.17
400 mg	2.00	-

Other

▪ RT-PCR

RT-PCR amplification for canine PPAR gamma (695-1130) or feline PPAR gamma (695-1130) was performed by use of ReverTra Ace (TOYOBO) on total RNA extracted from canine or feline adipose tissue using QuickGene system.



M : Marker (100 bp DNA Ladder : TOYOBO)
 1 : Canine PPAR gamma (695-1130)
 2 : Feline PPAR gamma (695-1130)

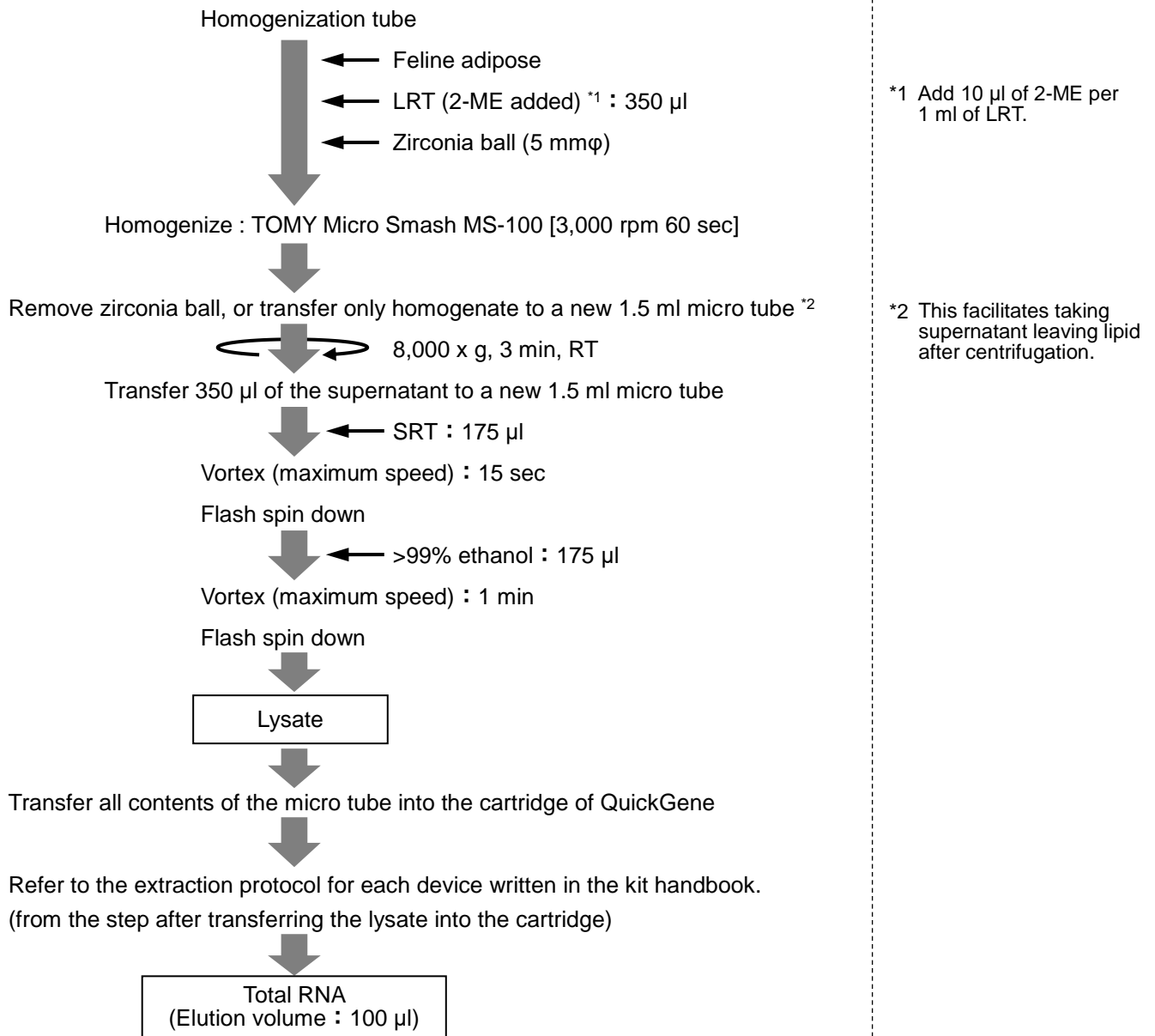
Common protocol is usable for the following

Canine Cutis, Feline Adipose Tissue

RA-b-2

Total RNA Extraction from Adipose Tissue of Feline

Protocol



Results

Total RNA was extracted from canine or feline adipose tissue.

The yield of total RNA

Amounts of tissue	QuickGene (μg)	Competitor A kit (μg)
30 mg	0.5	0.8
100 mg	2.3	-
200 mg	4.6	4.2
400 mg	28.0	-

Protein contamination : A260/280

Amounts of tissue	QuickGene	Competitor A kit
30 mg	1.88	1.58
100 mg	2.12	-
200 mg	2.16	2.17
400 mg	2.00	-

Other

RT-PCR

RT-PCR amplification for canine PPAR gamma (695-1130) or feline PPAR gamma (695-1130) was performed by use of ReverTra Ace (TOYOBO) on total RNA extracted from canine or feline adipose tissue using QuickGene system.



M : Marker (100 bp DNA Ladder : TOYOBO)
 1 : Canine PPAR gamma (695-1130)
 2 : Feline PPAR gamma (695-1130)

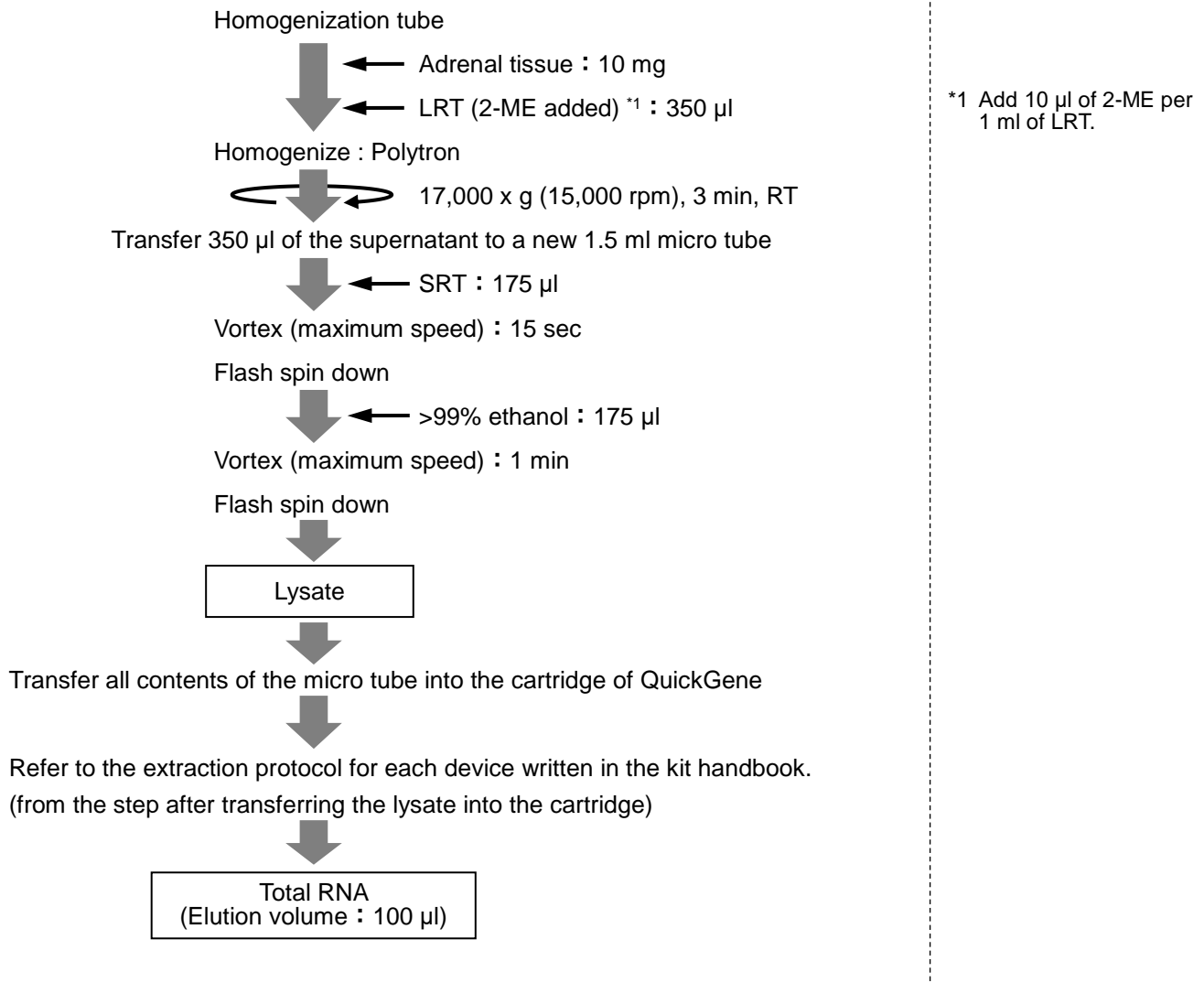
Common protocol is usable for the following

Canine Cutis, Canine Adipose Tissue

RA-b-3

Total RNA Extraction from Adrenal gland of Mouse

Protocol



Results

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

Amount of adrenal gland	Yield (µg)	A260/280
about 10 mg	1.0	1.5

Common protocol is usable for the following

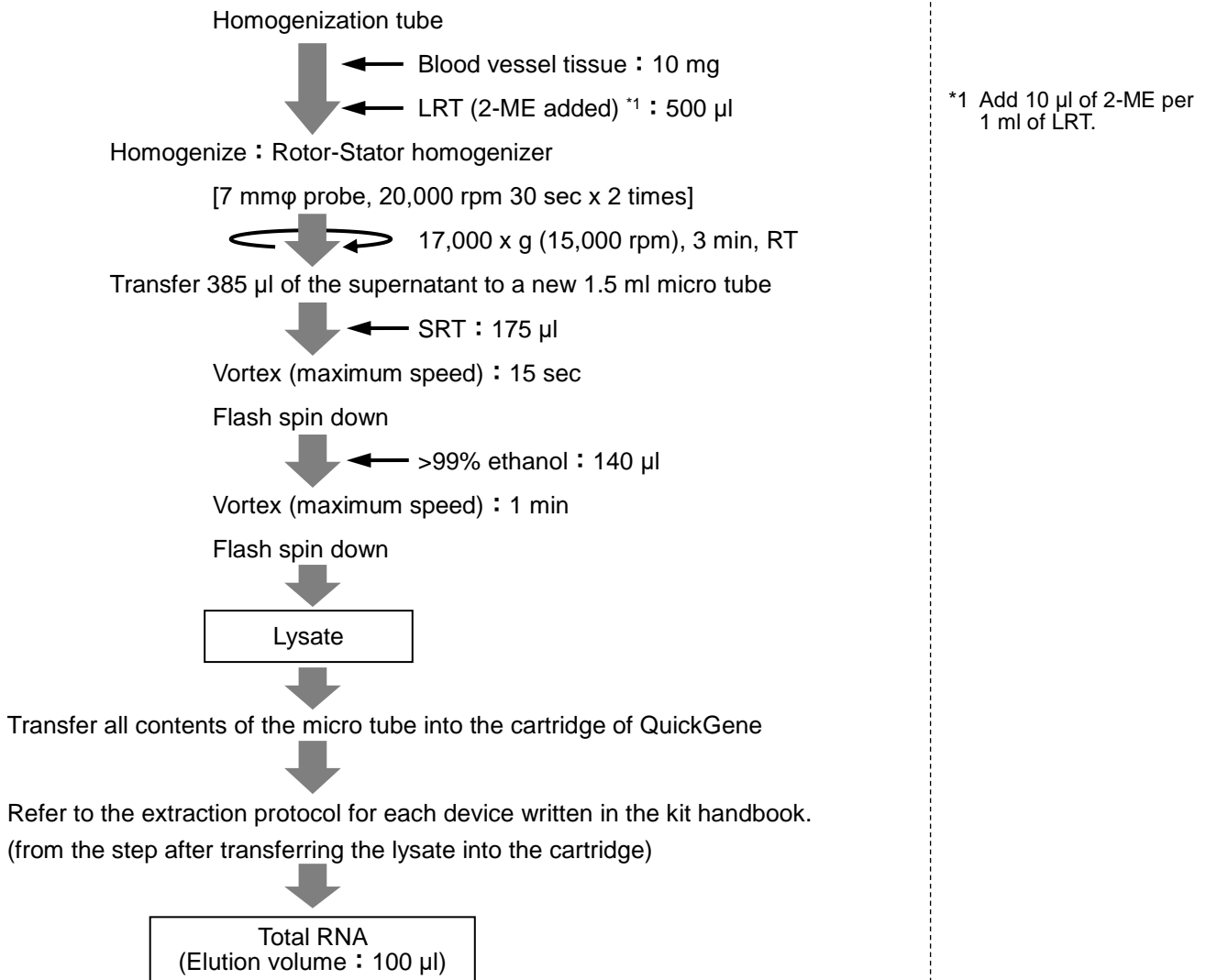
No Data

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).

RA-b-4

Total RNA Extraction from Blood vessel of Rabbit

Protocol



Results

The yield of total RNA

Amount of blood vessel	Yield (µg)
10 mg	1.0

Common protocol is usable for the following

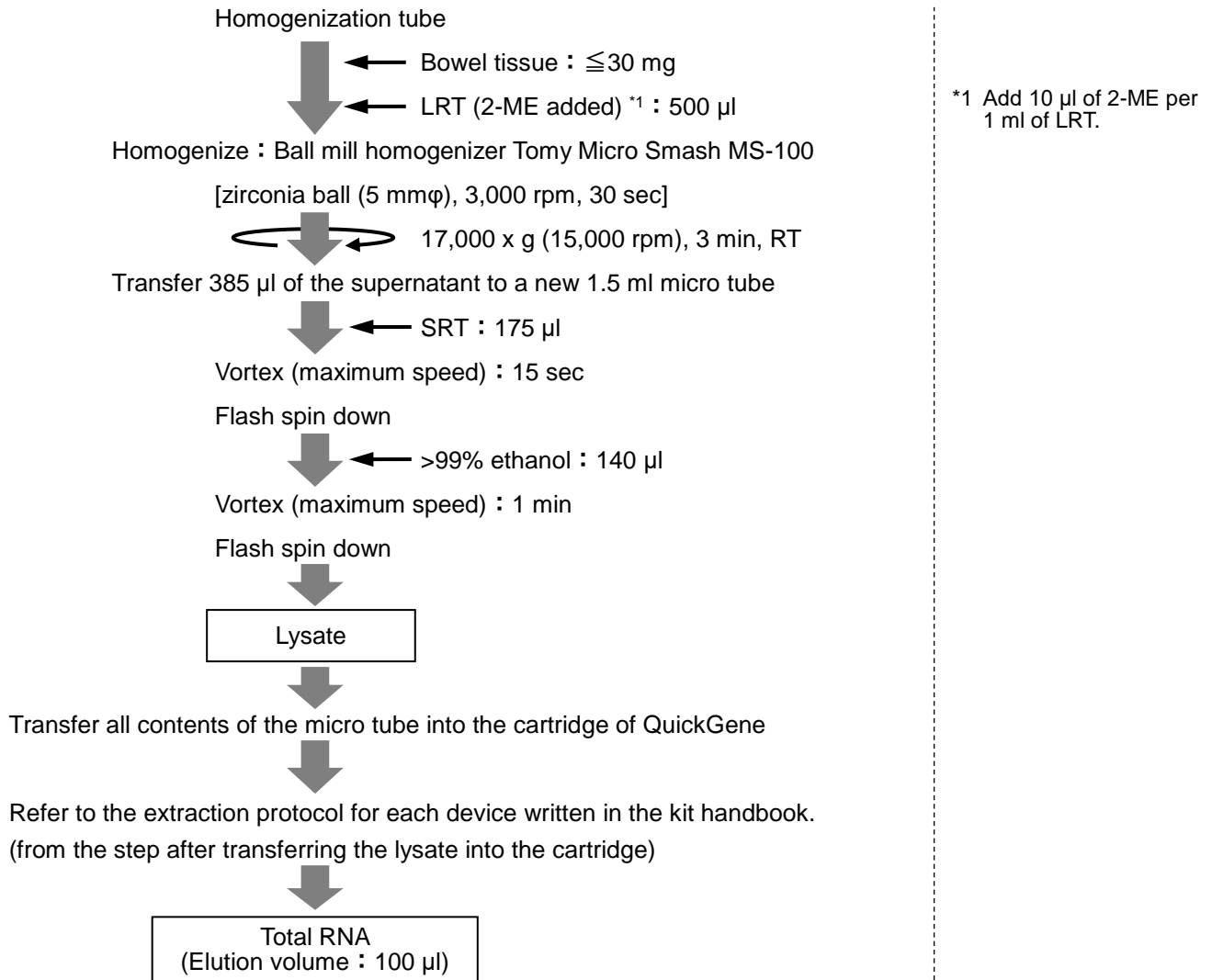
No Data

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).

RA-b-5

Total RNA Extraction from Bowel of Feline

Protocol



Results

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

Amount of bowel	Yield (μg)	A260/280
30 mg	13.8	1.78

Common protocol is usable for the following

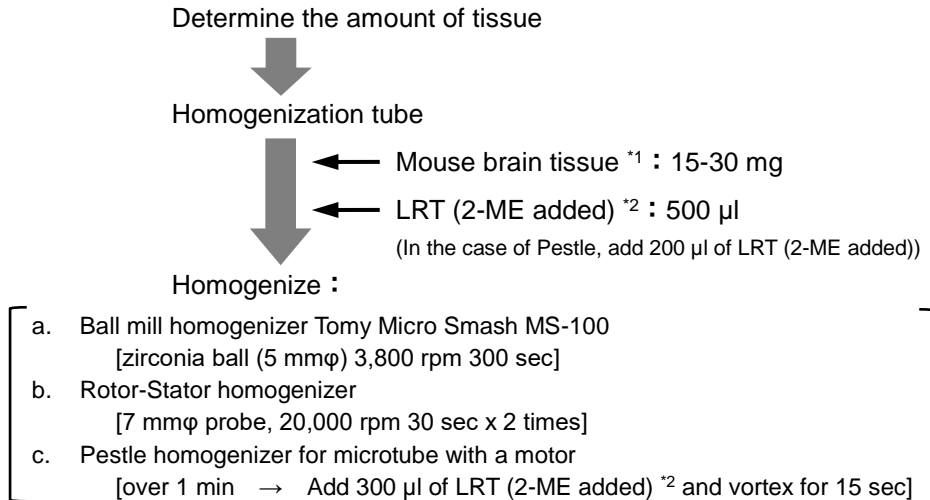
No Data

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).

RA-b-6

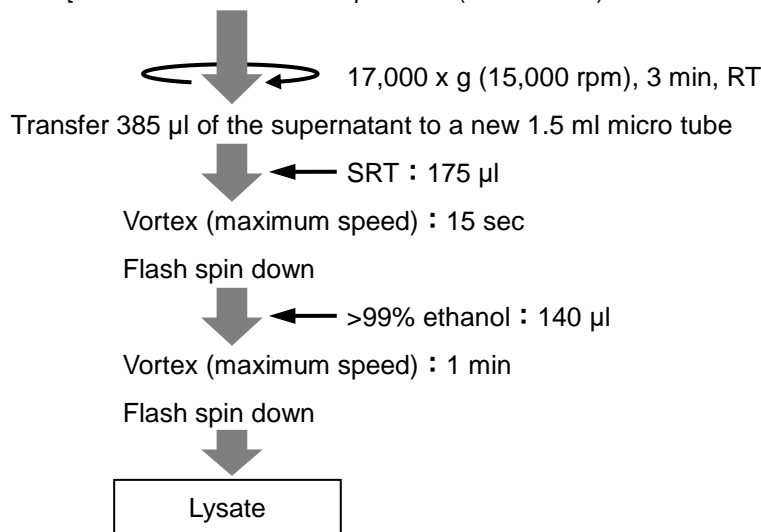
Total RNA Extraction from Brain of Mouse

Protocol 1 (15-30 mg)



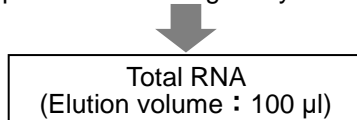
*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 μ l of 2-ME per 1 ml of LRT.

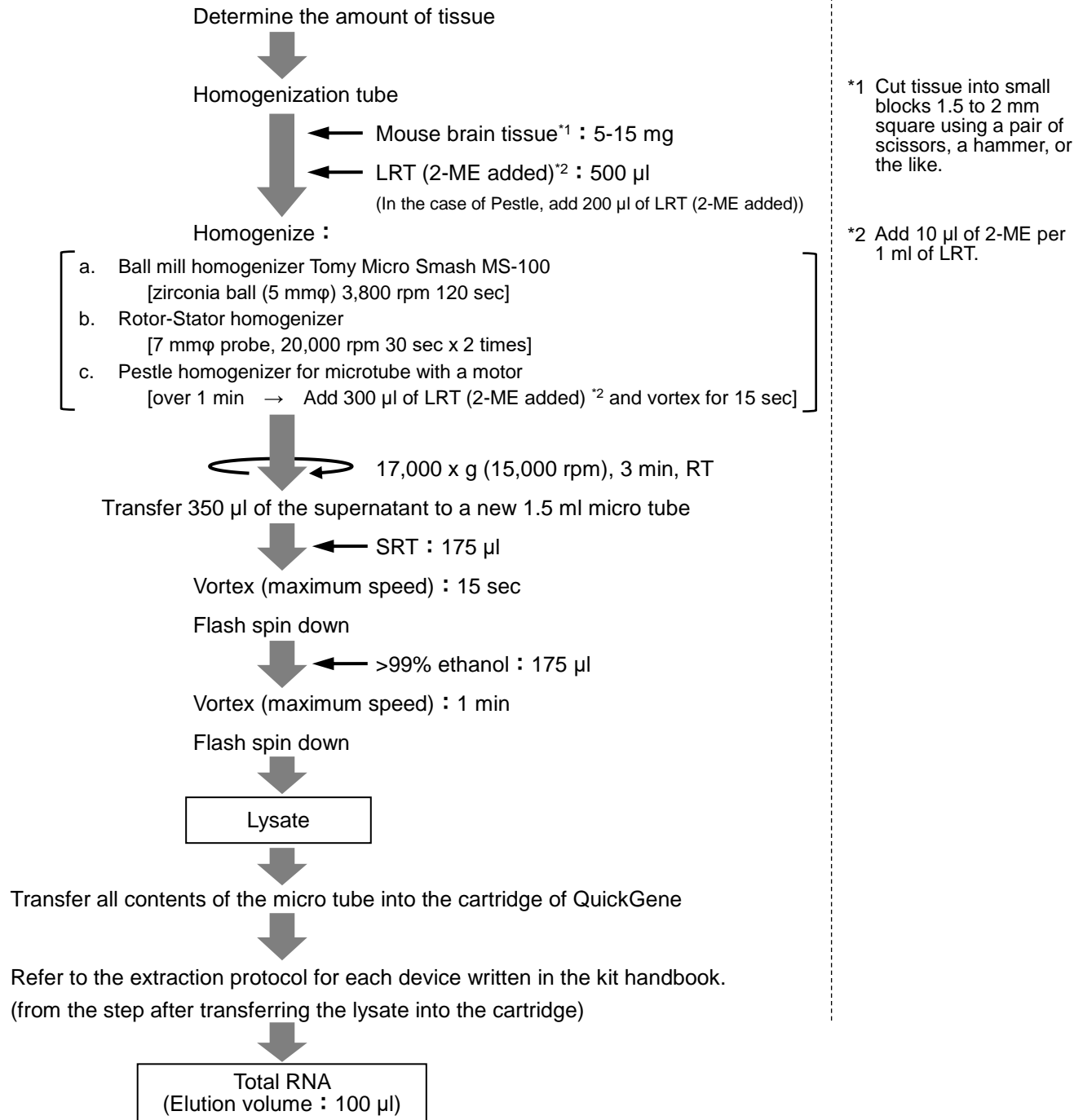


Transfer all contents of the micro tube into the cartridge of QuickGene

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



Protocol 2 (5-15 mg)

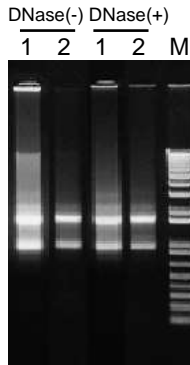


Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)

1 : QuickGene (with MS-100)

2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Brain	40 mg	21 µg	21 µg	40 mg	20 µg	21 µg

Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Brain	40 mg	2.11	2.17	2.11	1.95

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >

Template : Total RNA from mouse brain (with DNase treatment) 500 ng

Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)

Primer : G3PDH primer

Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)

1 : QuickGene

2 : Competitor A kit (spin column method)



Common protocol is usable for the following

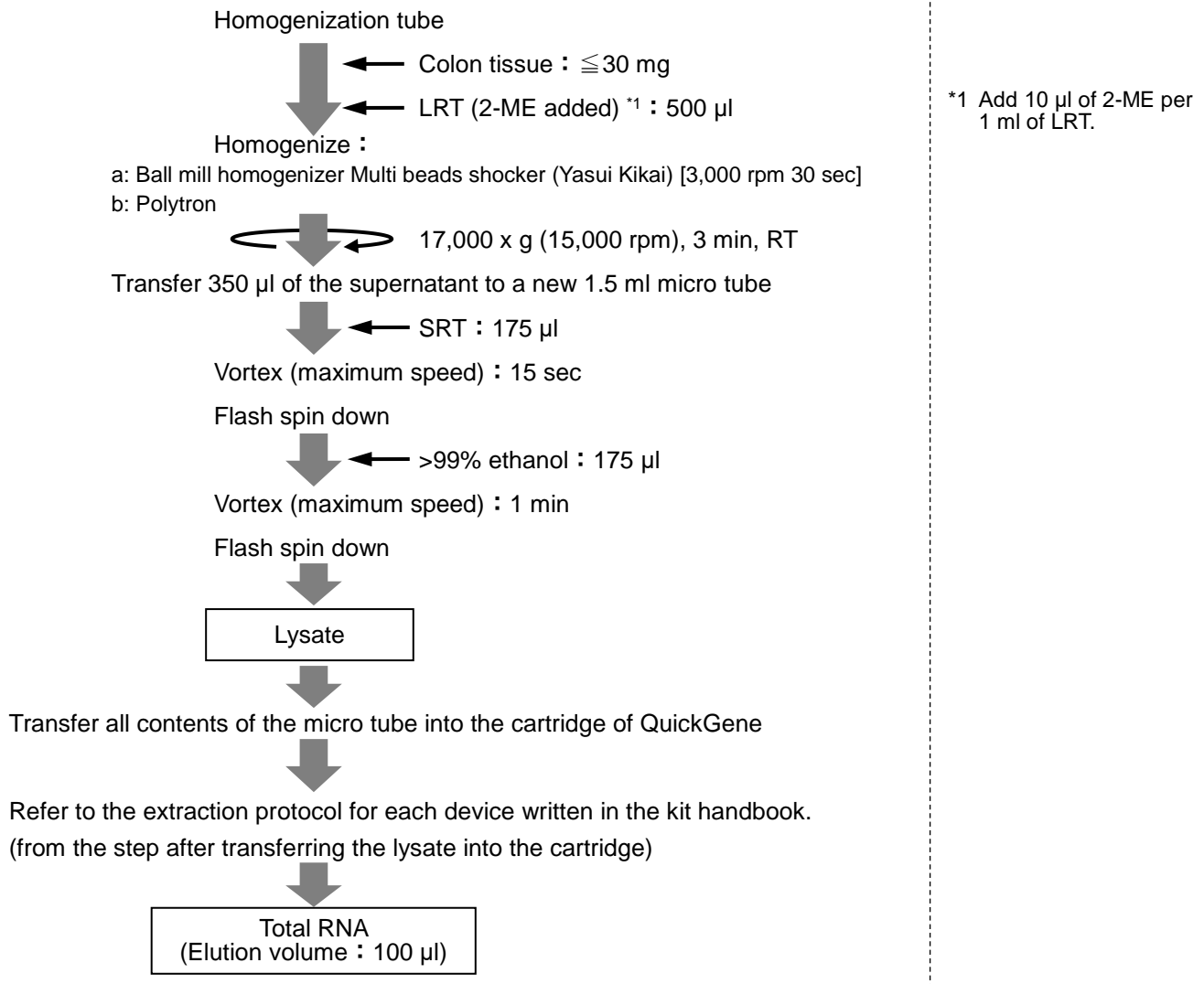
Mouse testis, Mouse Liver, Mouse Lung, Mouse Kidney, Mouse Spleen

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).

RA-b-7

Total RNA Extraction from Colon of Mouse

Protocol



Results

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

Amount of colon	Yield (μ g)	A260/280
a : about 5 mg	about 8.0	-
b : about 10 mg	3.0	2.7

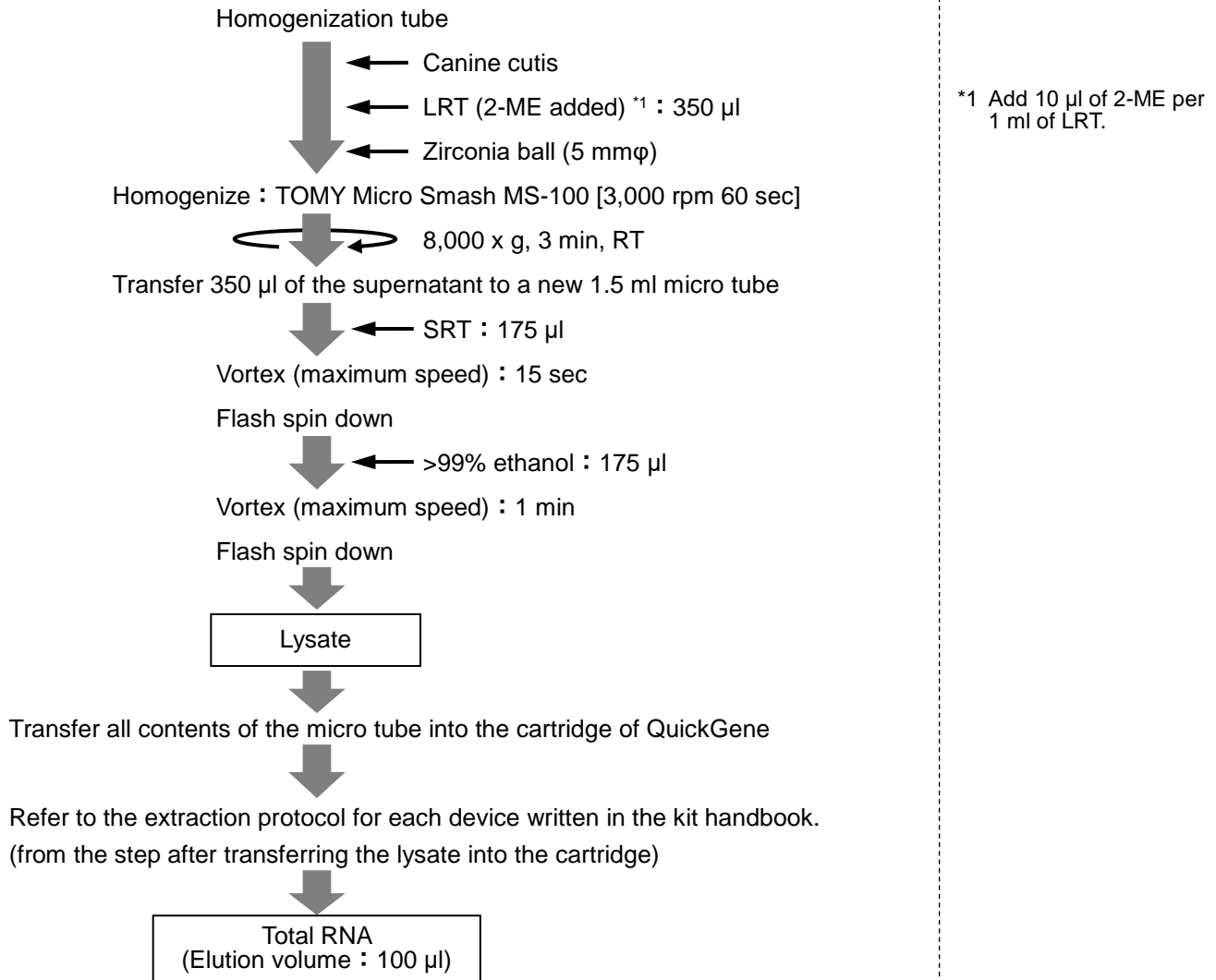
Common protocol is usable for the following

No Data

RA-b-8

Total RNA Extraction from Cutis of Canine

Protocol



Results

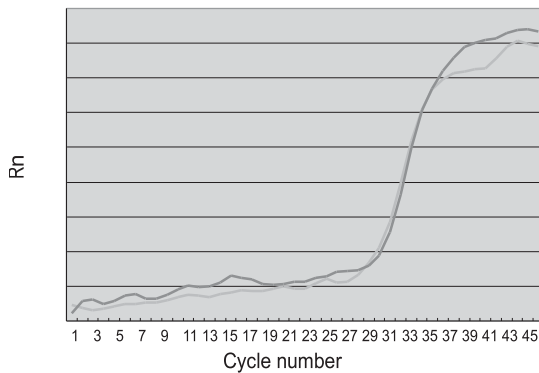
The yield of total RNA

Amounts of tissue	Yield (µg)	
	QuickGene	Competitor A kit
1 mm ²	below detection limit	below detection limit

Other

One-step Realtime RT-PCR

One-step Realtime RT-PCR was performed to amplify *GAPDH* by use of QuantiTect Probe RT-PCR kit (QIAGEN) and ABI PRISM7000 Sequence Detection System (Applied Biosystems) with total RNA extracted from canine cutis.



Although the yield of total RNA was below detection limit for measurement with absorptiometer, one-step Realtime RT-PCR showed excellent results.

* Both are data for total RNA extracted with QuickGene system.

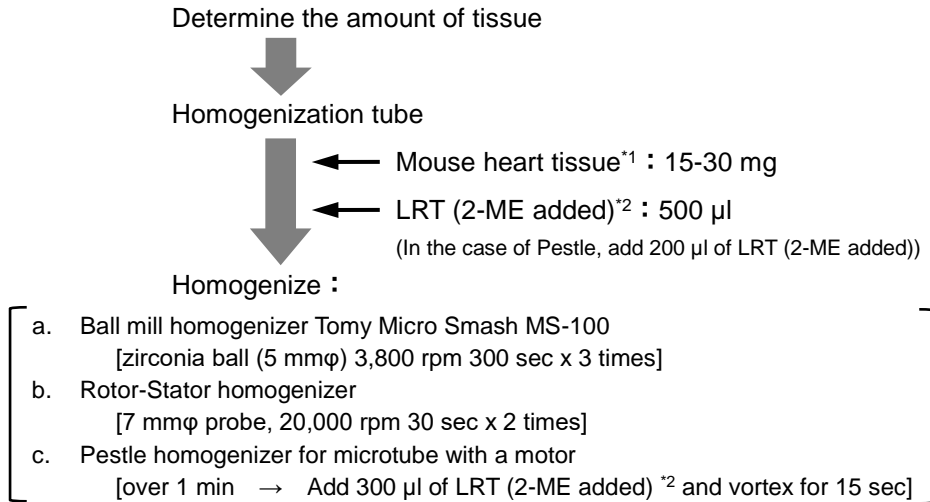
Common protocol is usable for the following

Feline Adipose Tissue, Canine Adipose Tissue

RA-b-9

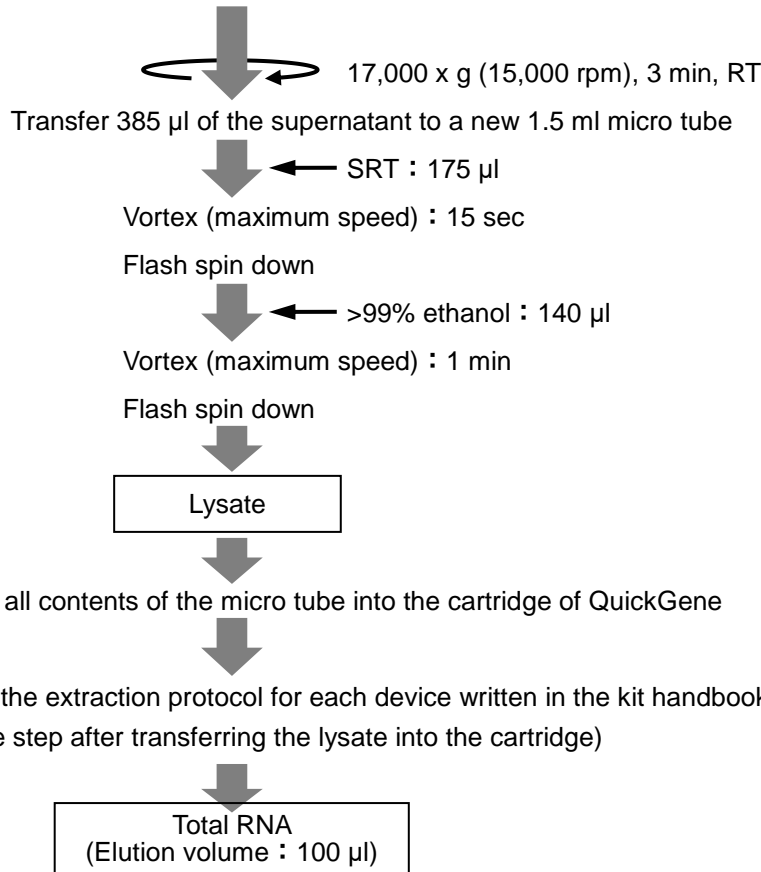
Total RNA Extraction from Heart of Mouse

Protocol 1 (15-30 mg)

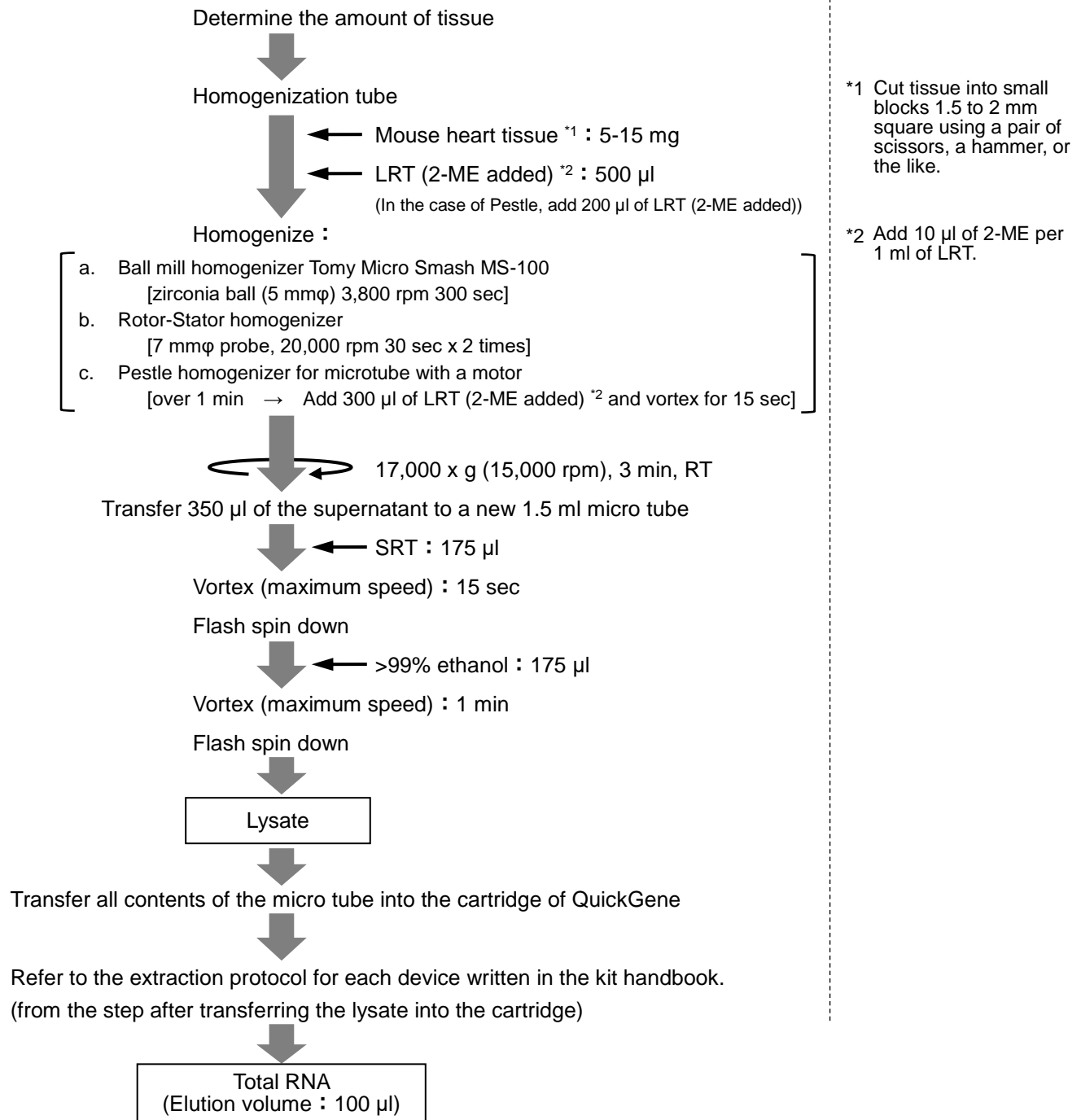


*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 μ l of 2-ME per 1 ml of LRT.



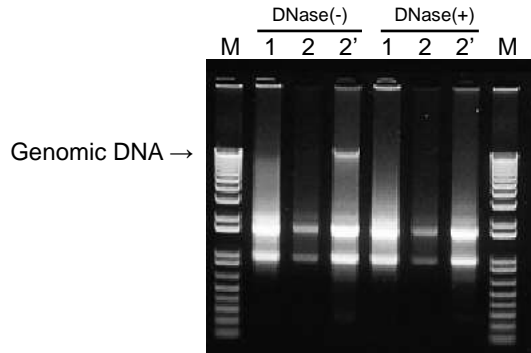
Protocol 2 (5-15 mg)



Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA.
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)
2' : Competitor A kit (spin column method, for Fibrous)

For heart, QuickGene system enables extraction of total RNA with genomic DNA contamination less than that in the case of Competitor A kit (spin column method).

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Heart	30 mg	21 µg	23 µg	5 mg	4 µg	4 µg

Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

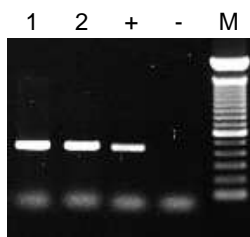
Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Heart	30 mg	2.37	2.33	2.18	2.16

(with Ball mill homogenizer)

Other

RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >

Template : Total RNA from mouse heart (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

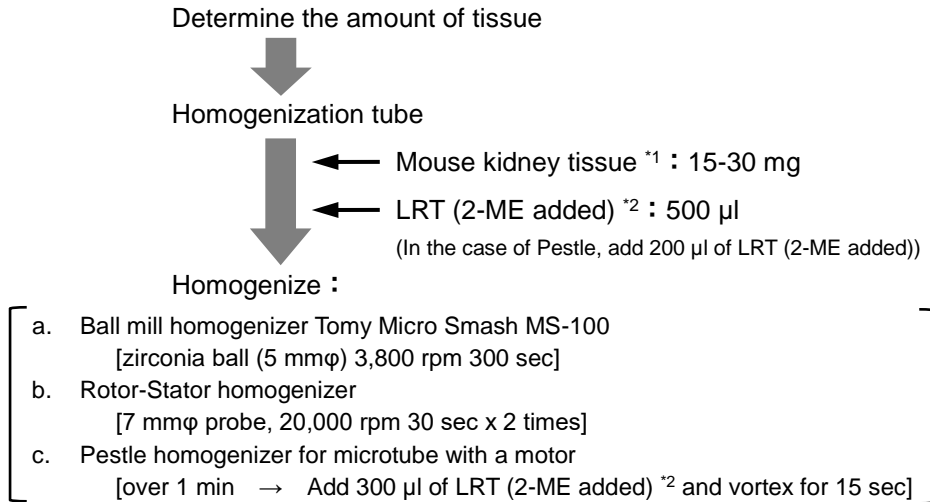
Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Lung, Mouse Kidney, Mouse Spleen

RA-b-10

Total RNA Extraction from Kidney of Mouse

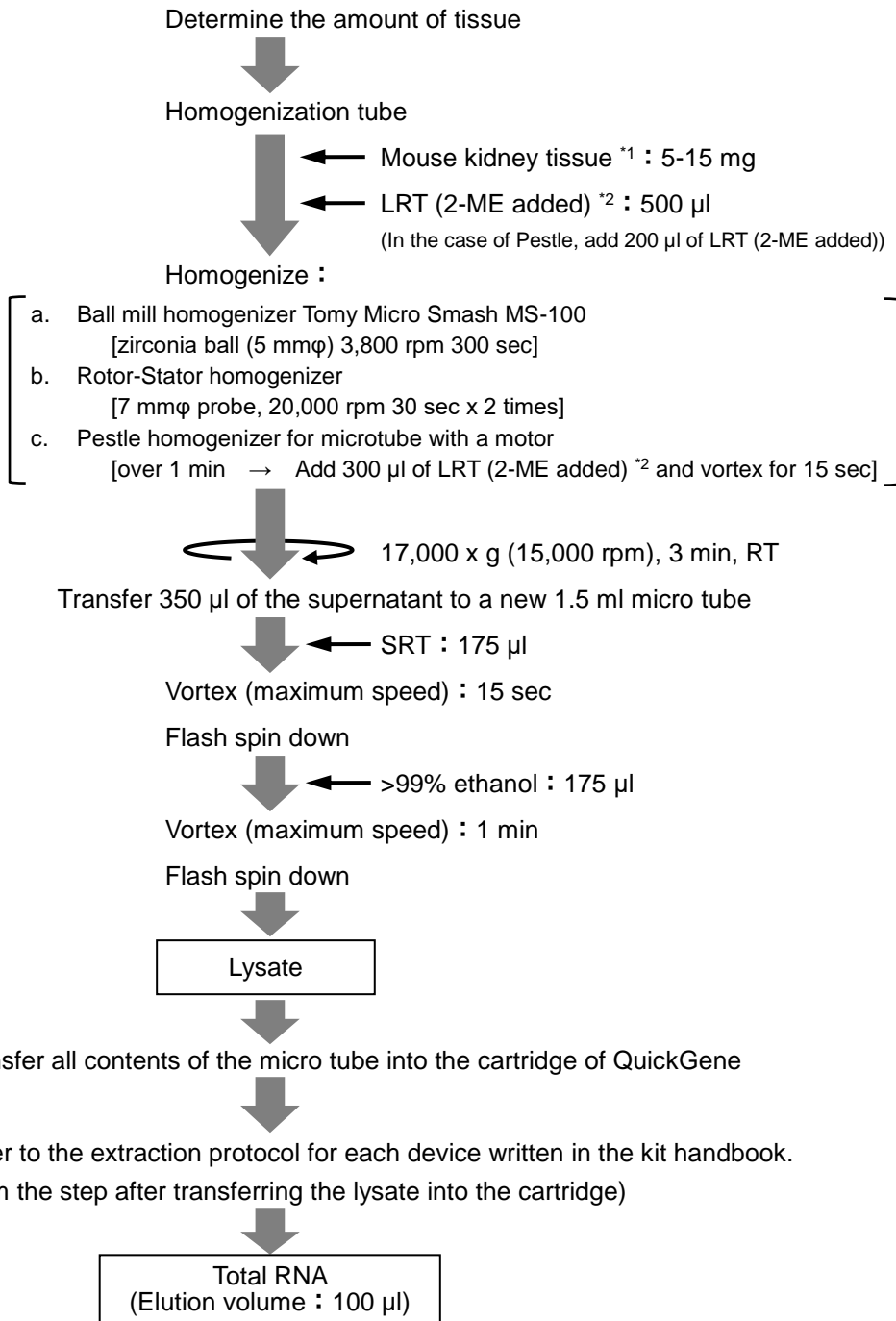
Protocol 1 (15-30 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

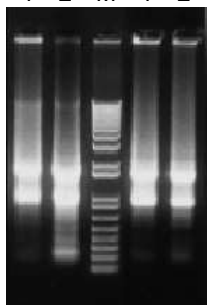
Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

Electrophoresis conditions : 1% Agarose / 1 x TAE

DNase(-) DNase(+)
1 2 M 1 2



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)

1 : QuickGene (with MS-100)

2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Kidney	30 mg	55 µg	54 µg	5 mg	16 µg	13 µg

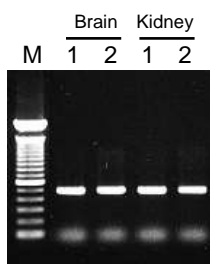
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Kidney	30 mg	2.30	2.17	2.21	2.09

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >

Template : Total RNA from mouse brain and kidney (with DNase treatment) 500 ng

Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)

Primer : *G3PDH* primer

Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE

M : Marker (100 bp DNA Ladder : Invitrogen)

1 : QuickGene

2 : Competitor A kit (spin column method)

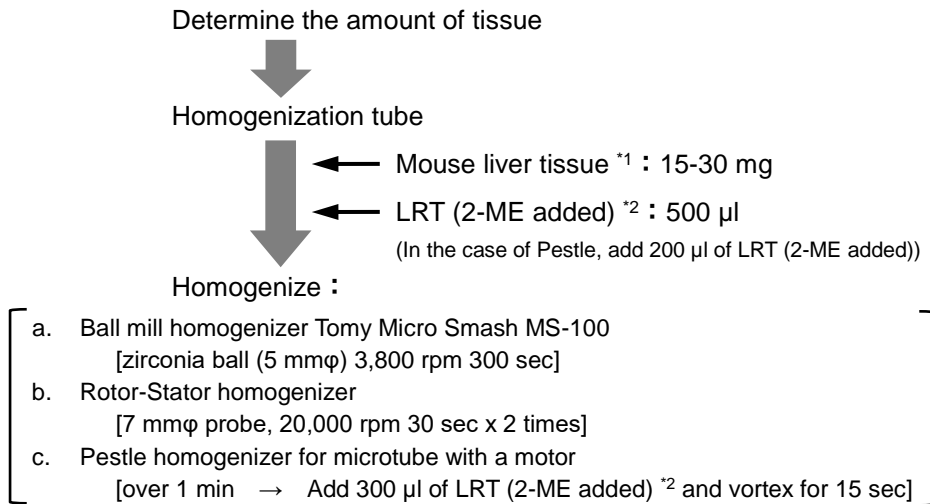
Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Spleen

RA-b-11

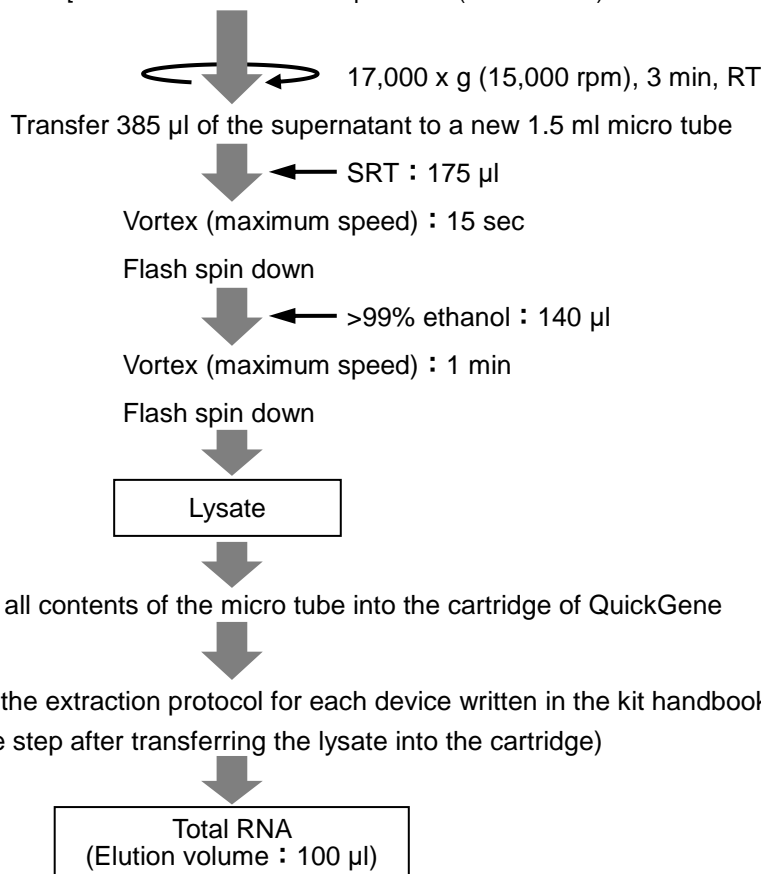
Total RNA Extraction from Liver of Mouse

Protocol 1 (15-30 mg)

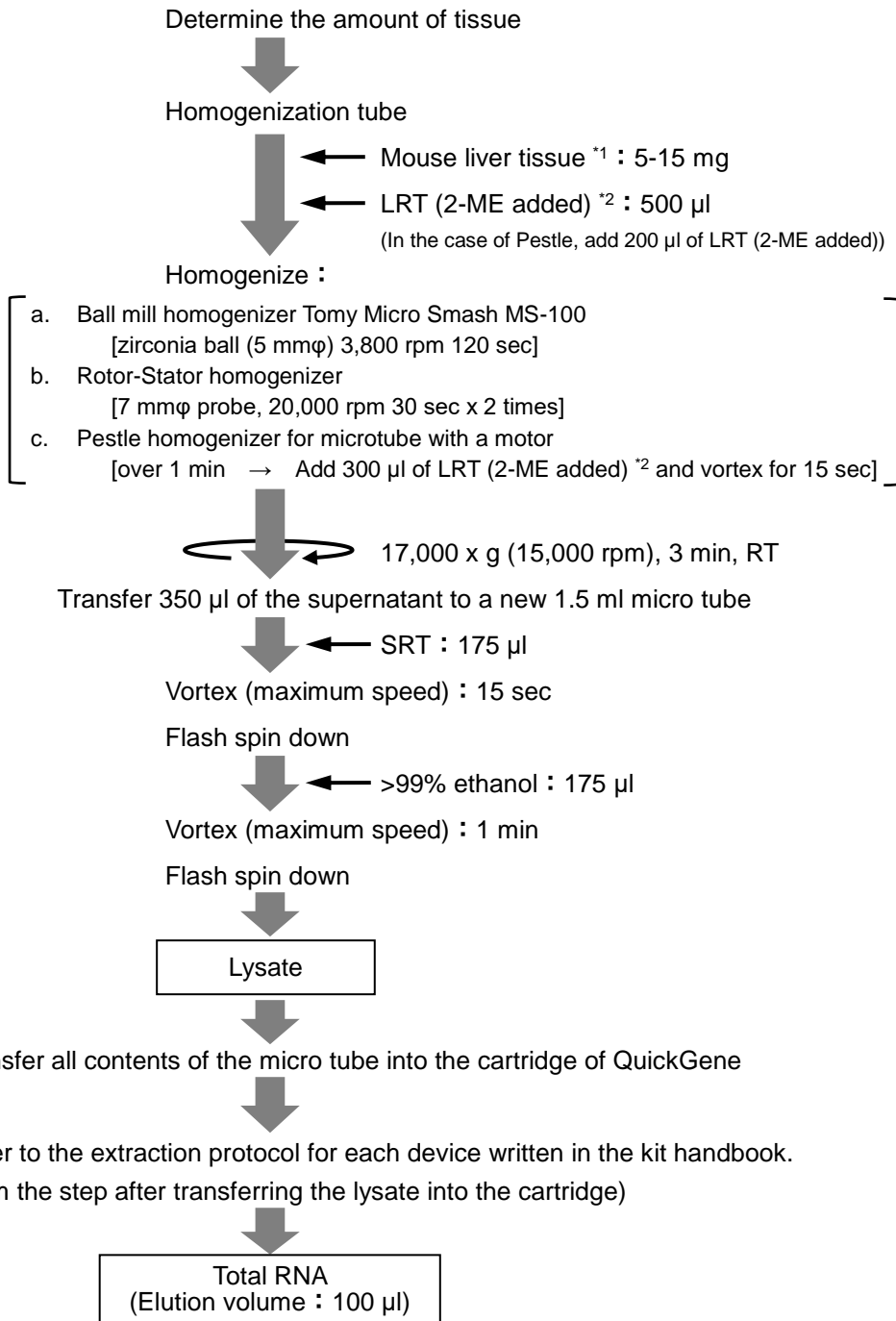


*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 μ l of 2-ME per 1 ml of LRT.



Protocol 2 (5-15 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

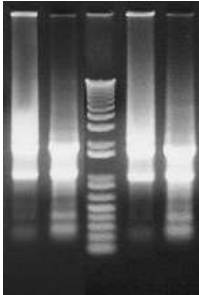
Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

Electrophoresis conditions : 1% Agarose / 1 x TAE

DNase(-) DNase(+)
1 2 M 1 2



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Liver	5 mg	23 µg	25 µg	5 mg	33 µg	27 µg
	30 mg	122 µg	142 µg	15 mg	54 µg	55 µg

Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Liver	5 mg	2.24	2.18	2.06	1.99
	30 mg	2.21	2.20	2.21	2.26

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

< RT reaction conditions >

Template : Total RNA from mouse tissue (with DNase treatment) 500 ng

Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)

Primer : *G3PDH* primer

Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE

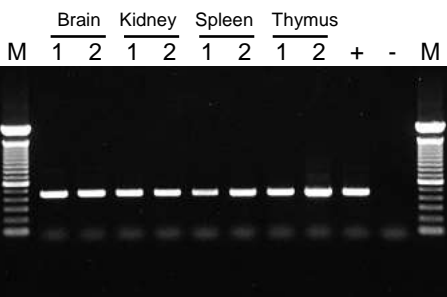
M : Marker (100 bp DNA Ladder : Invitrogen)

1 : QuickGene

2 : Competitor A kit (spin column method)

+ : Positive control (mLiver RNA : Clontech)

- : Negative control (RNase-free water)



Common protocol is usable for the following

Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Spleen

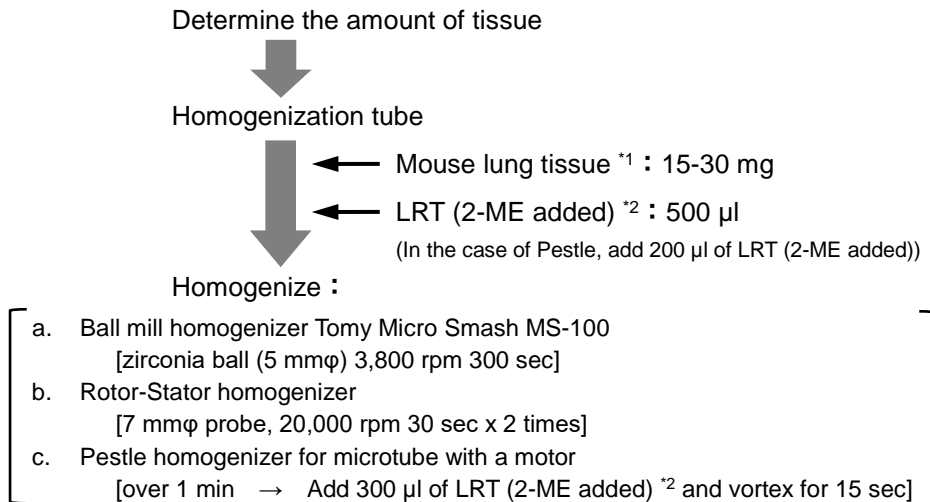
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.

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

RA-b-12

Total RNA Extraction from Lung of Mouse

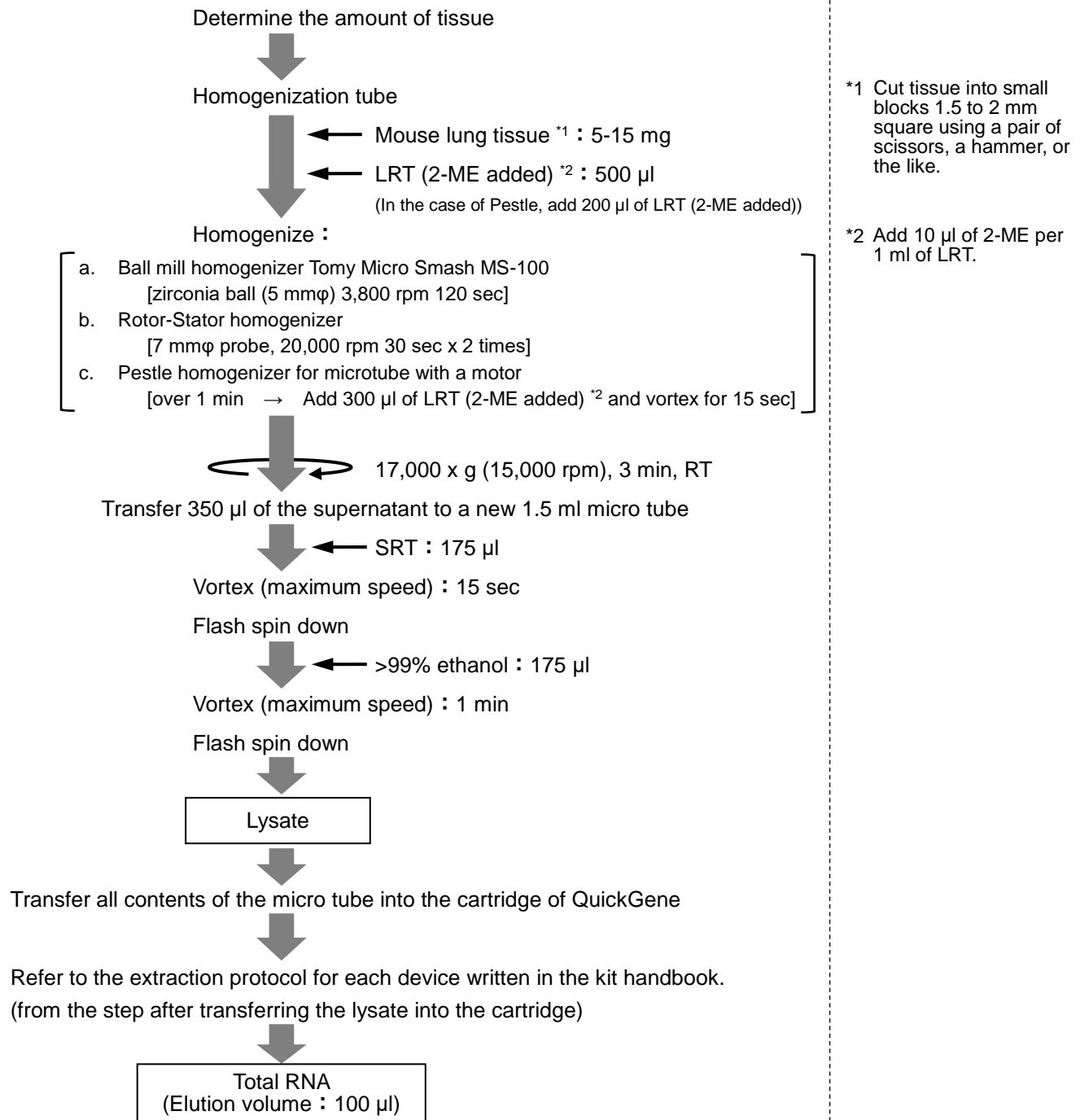
Protocol 1 (15-30 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)



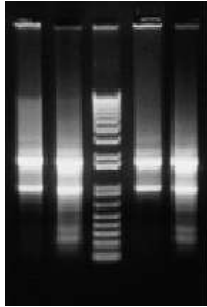
Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

Electrophoresis conditions : 1% Agarose / 1 x TAE

DNase(-) DNase(+)
1 2 M 1 2



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Lung	30 mg	29 µg	28 µg	15 mg	7 µg	7 µg

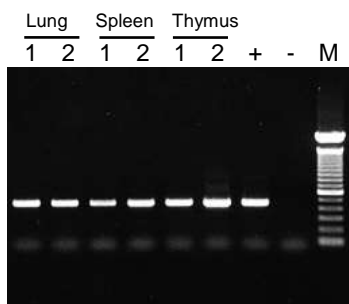
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Lung	30 mg	2.18	2.19	2.16	2.05

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >

Template : Total RNA from mouse tissue (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

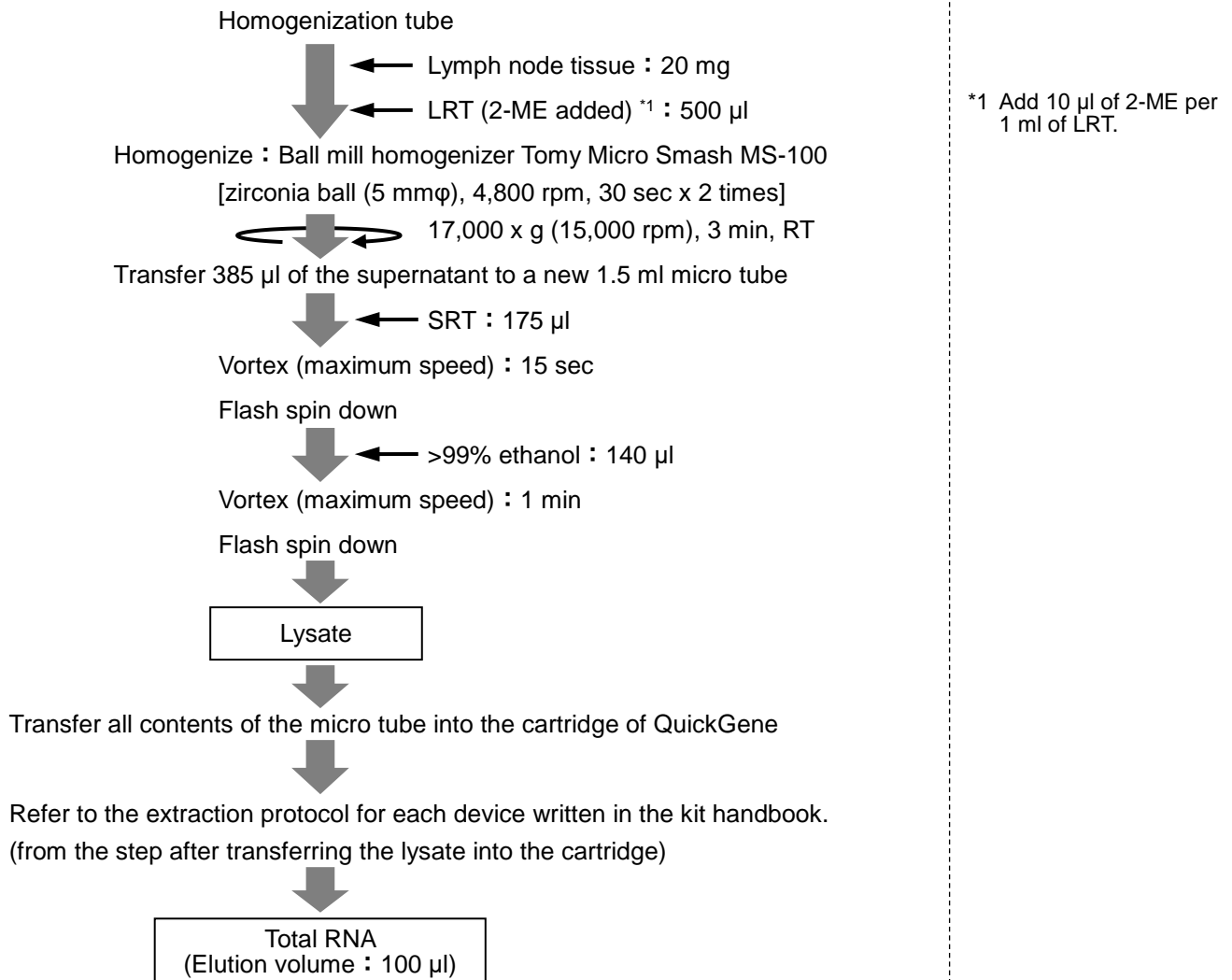
Mouse testis, Mouse Liver, Mouse Brain, Mouse Kidney, Mouse Spleen

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).

RA-b-13

Total RNA Extraction from Lymph node of Mouse

Protocol



Results

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

Amount of bowel	Yield (μ g)	A260/280
20 mg	6.8	2.0

Common protocol is usable for the following

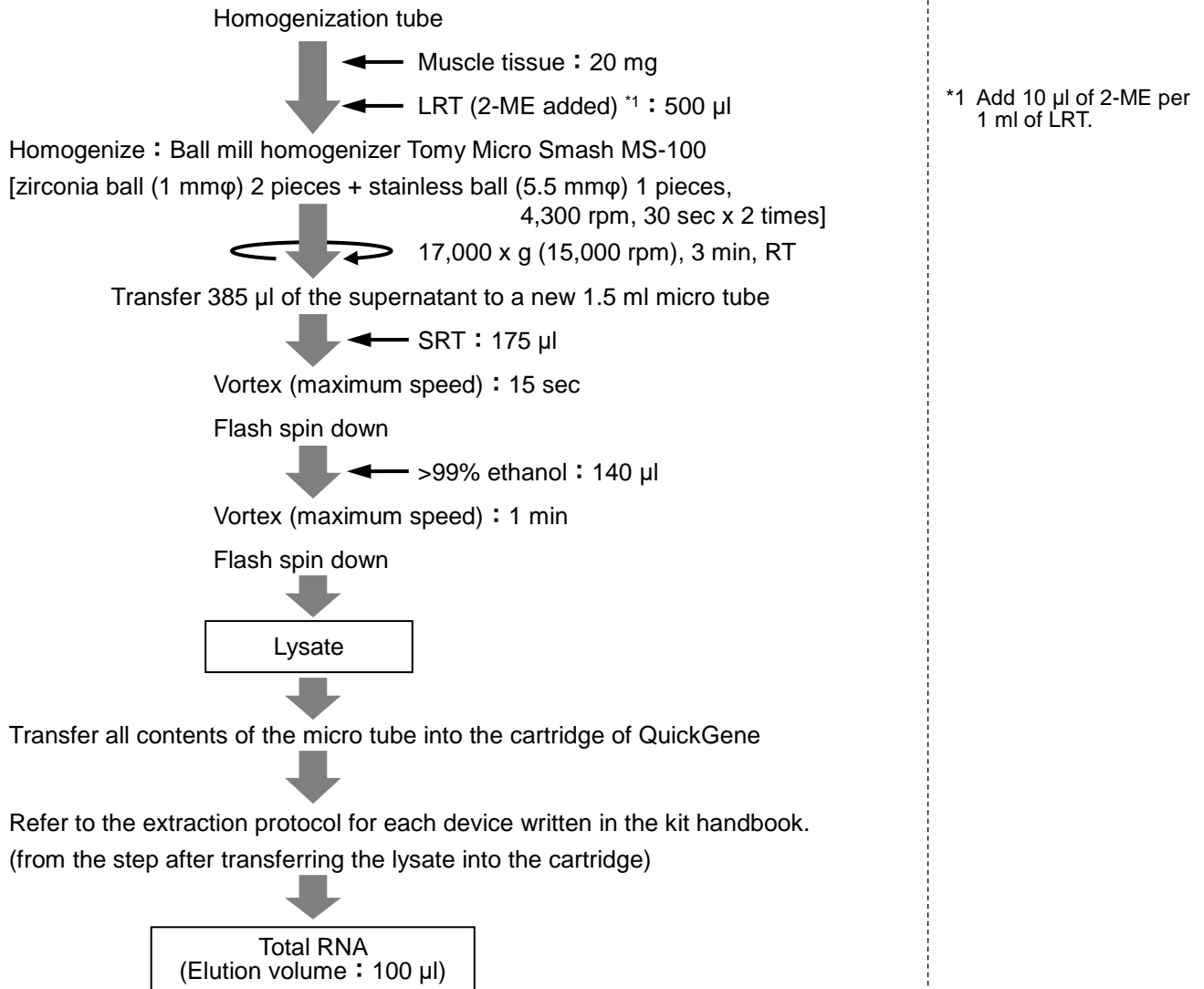
No Data

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).

RA-b-14

Total RNA Extraction from Muscle of Rat

Protocol



Results

The yield of total RNA

Amount of bowel	Yield (µg)
8.8 mg	2.0

Common protocol is usable for the following

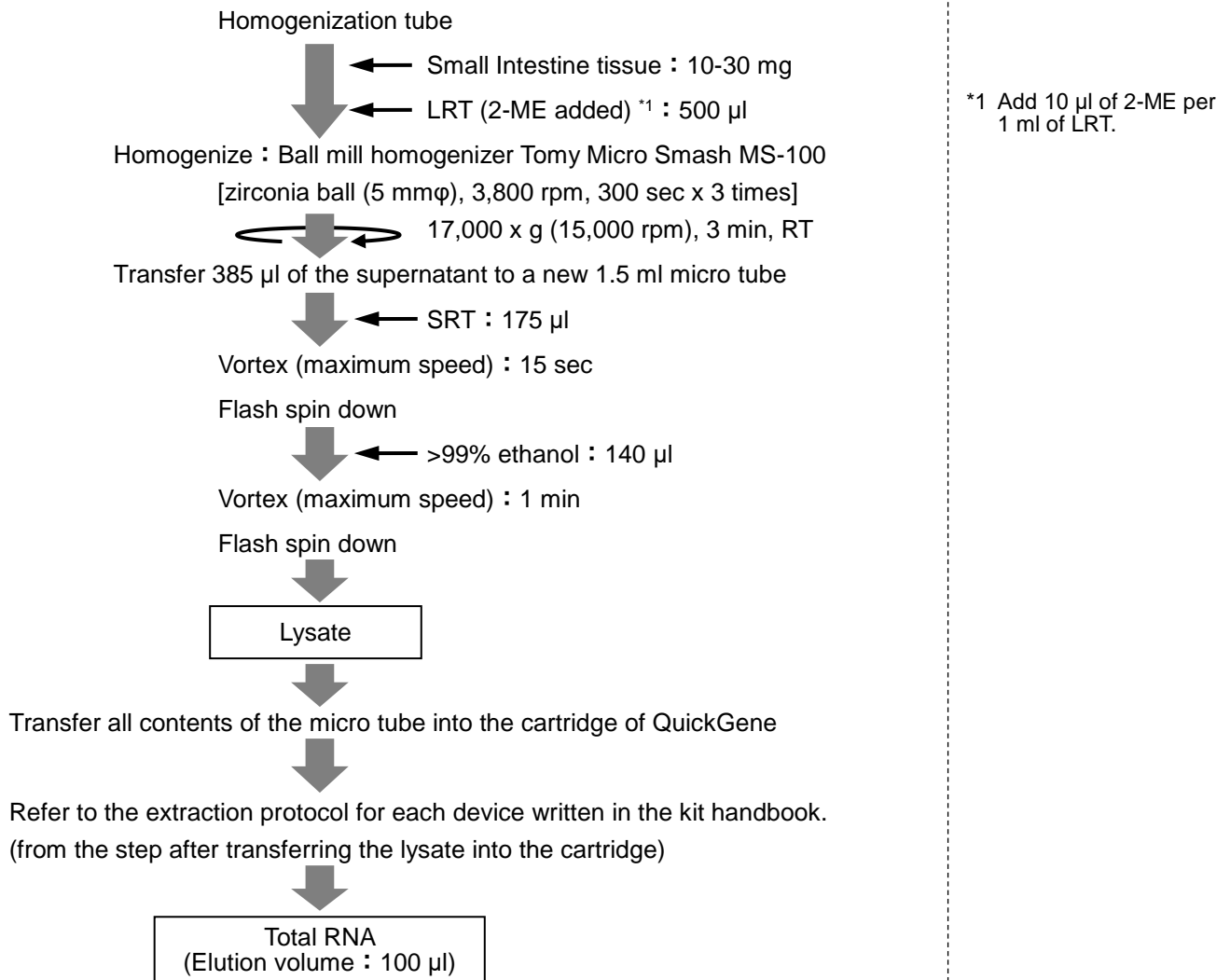
No Data

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).

RA-b-15

Total RNA Extraction from Small Intestine of Mouse

Protocol



Results

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

Amount of small intestine	Yield (μ g)	A260/280
14.7 mg	4.4	2.01

Common protocol is usable for the following

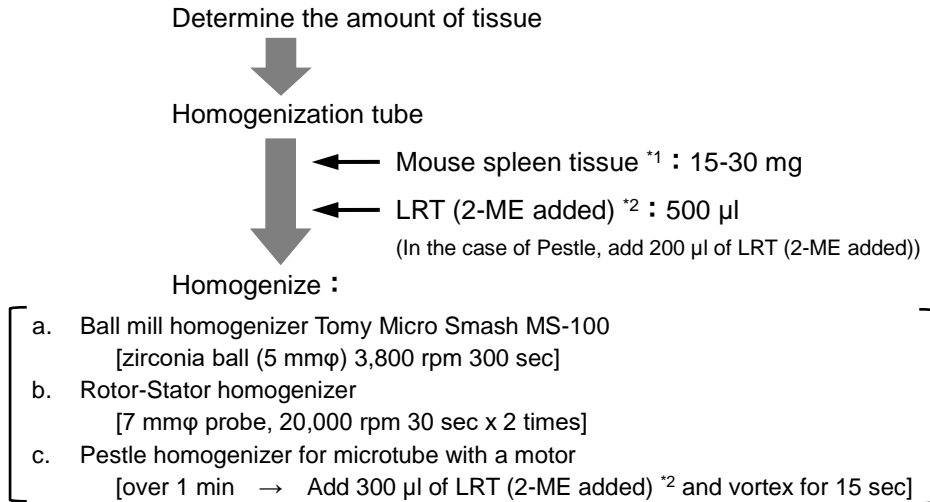
Mouse Heart

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).

RA-b-16

Total RNA Extraction from Spleen of Mouse

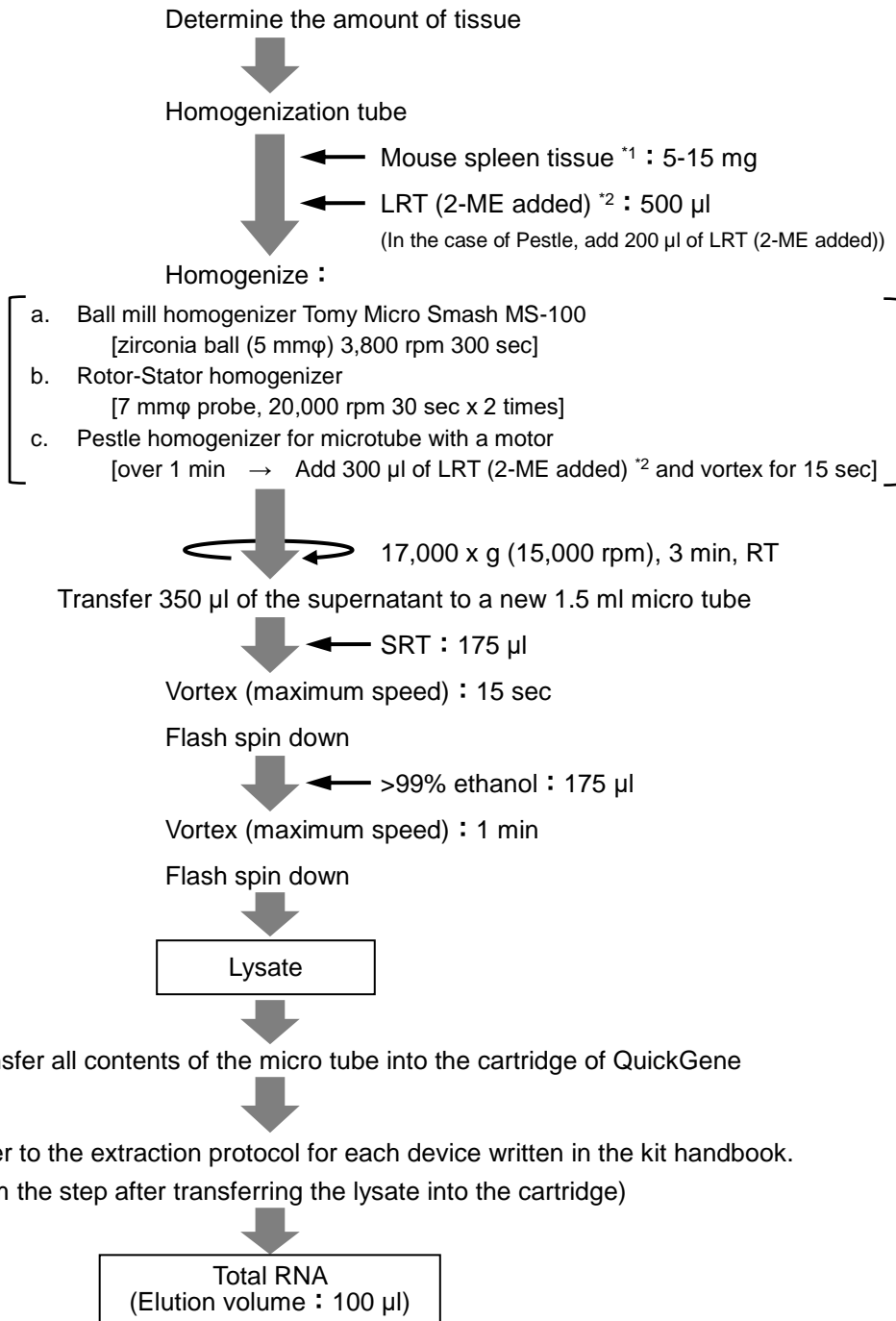
Protocol 1 (15-30 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Protocol 2 (5-15 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

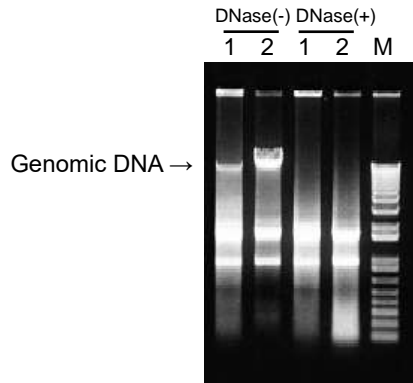
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA extracted from various tissues of mouse using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).

Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
 1 : QuickGene (with MS-100)
 2 : Competitor A kit (spin column method)

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Spleen	30 mg	48 µg	54 µg	20 mg	32 µg	31 µg

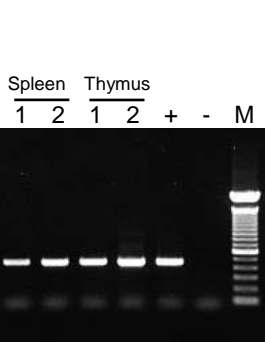
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Spleen	30 mg	2.05	2.30	2.23	2.09

Other

▪ RT-PCR

RT-PCR was performed on total RNA extracted using QuickGene system (with Ball mill homogenizer) and Competitor A kit (spin column method).



< RT reaction conditions >

Template : Total RNA from mouse spleen and thymus (with DNase treatment) 500 ng
 Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)
 Primer : *G3PDH* primer
 Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE
 M : Marker (100 bp DNA Ladder : Invitrogen)
 1 : QuickGene
 2 : Competitor A kit (spin column method)
 + : Positive control (mLiver RNA : Clontech)
 - : Negative control (RNase-free water)

Common protocol is usable for the following

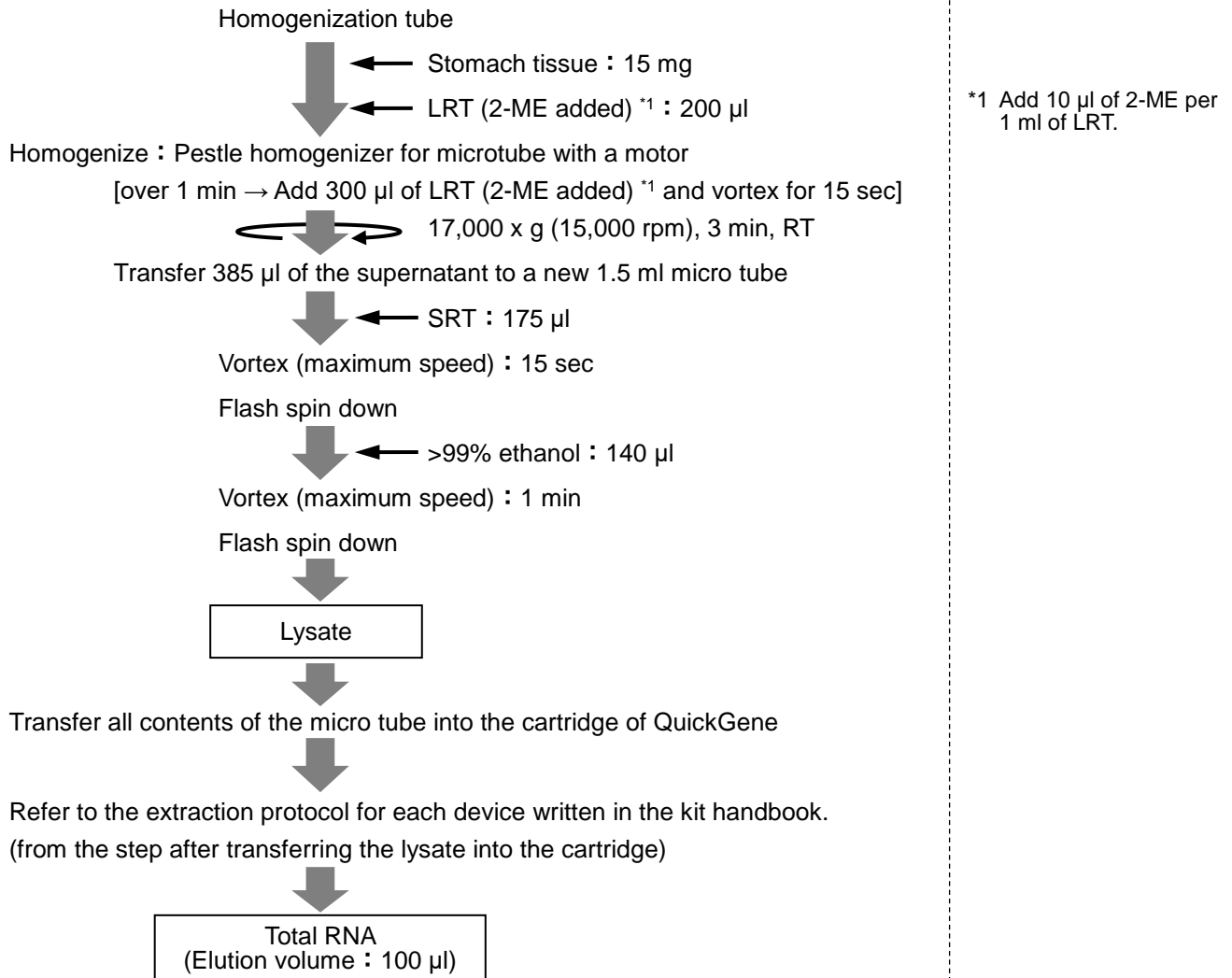
Mouse testis, Mouse Liver, Mouse Brain, Mouse Lung, Mouse Kidney

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).

RA-b-17

Total RNA Extraction from Stomach of Human

Protocol



Results

The yield of total RNA

Amount of stomach	Yield (µg)
15 mg	2.0

Common protocol is usable for the following

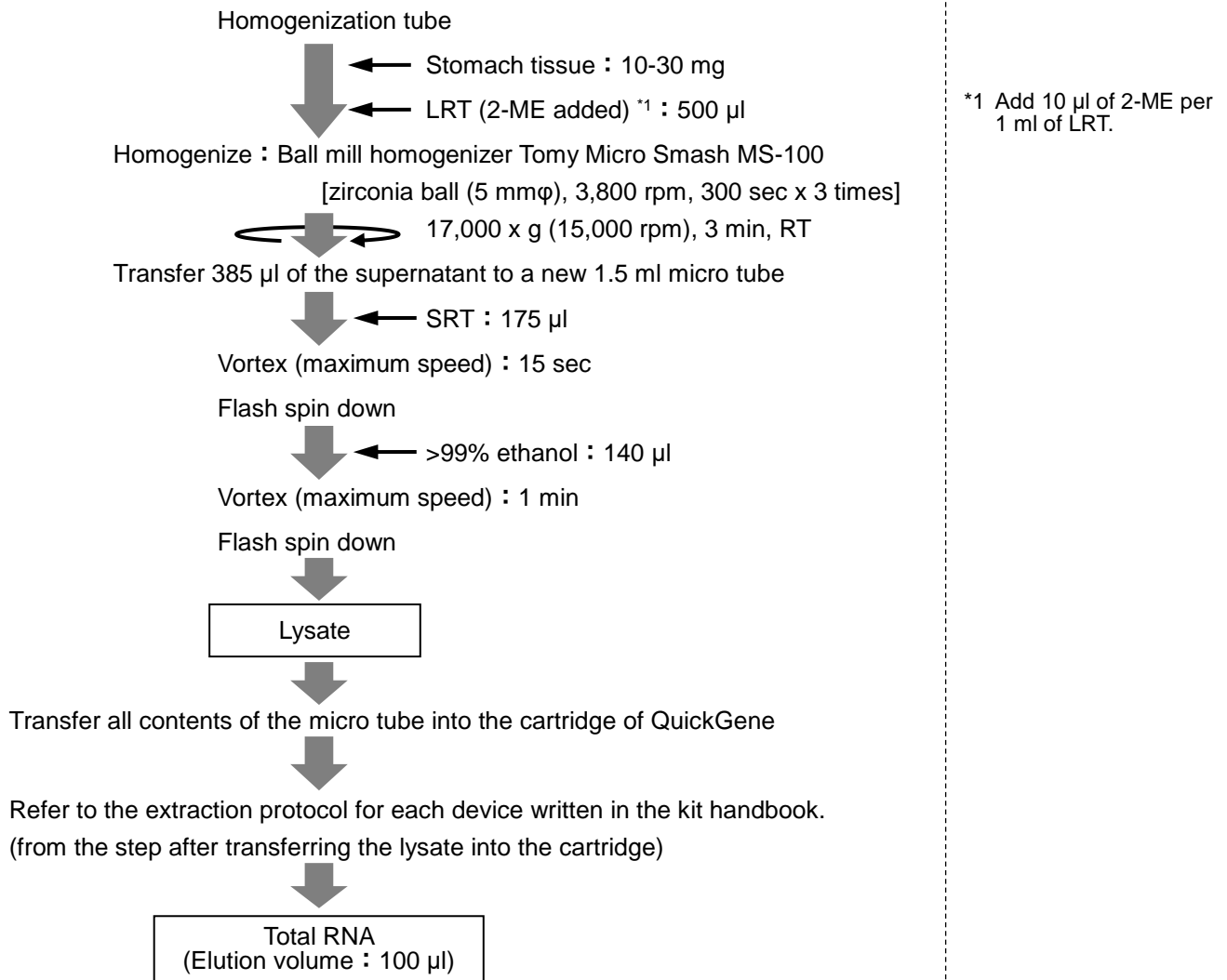
No Data

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).

RA-b-18

Total RNA Extraction from Stomach of Mouse

Protocol



Results

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

Amount of stomach	Yield (μ g)	A260/280
11.1 mg	12.6	2.06

Common protocol is usable for the following

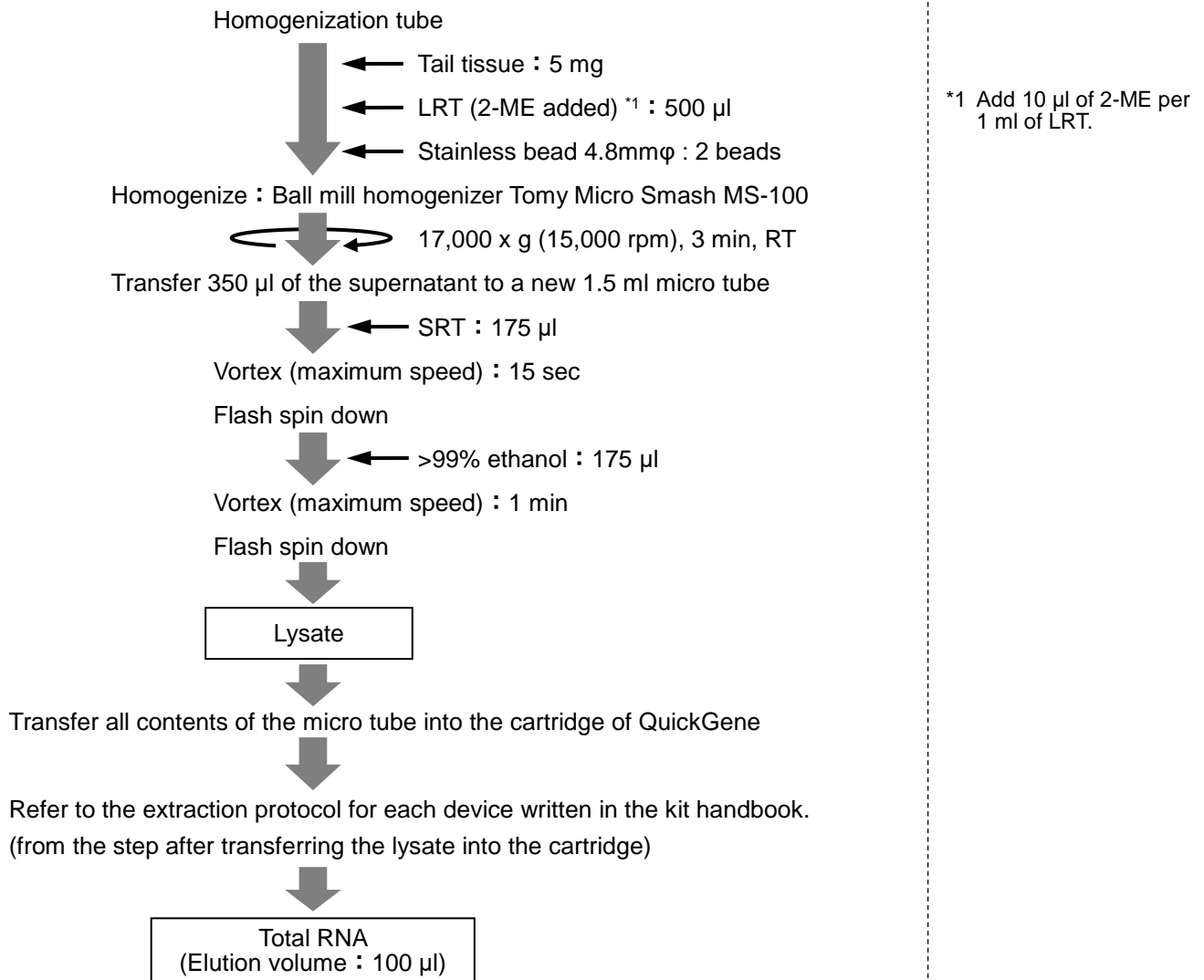
Mouse Heart

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).

RA-b-19

Total RNA Extraction from Tail of Mouse

Protocol



Results

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

Amount of tail	Yield (μ g)	A260/280
about 5 mg	4.0	2.36

Common protocol is usable for the following

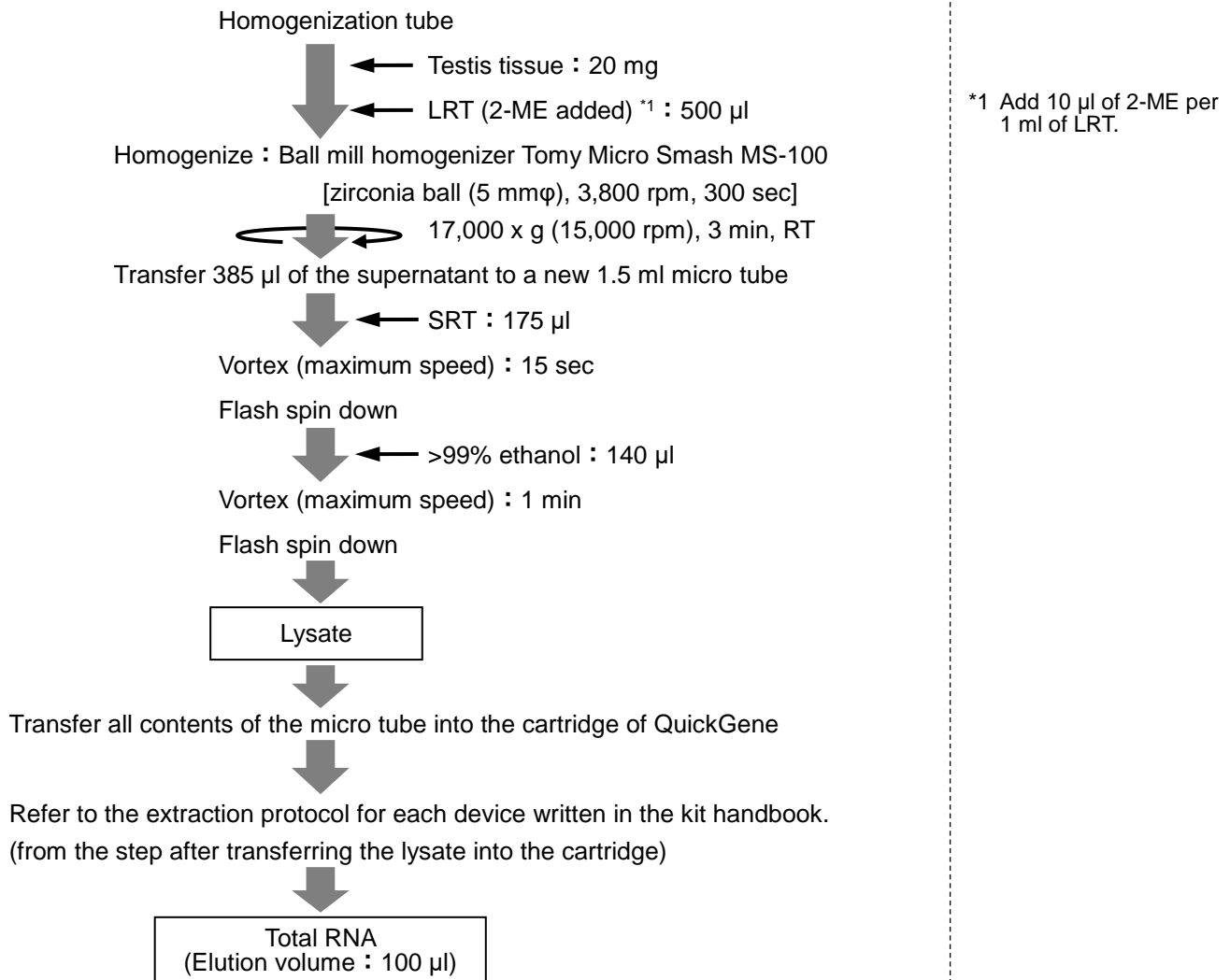
No Data

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).

RA-b-20

Total RNA Extraction from Testis of Mouse

Protocol



Results

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

Amount of testis	Yield (μ g)	A260/280
20 mg	20	2.0

Common protocol is usable for the following

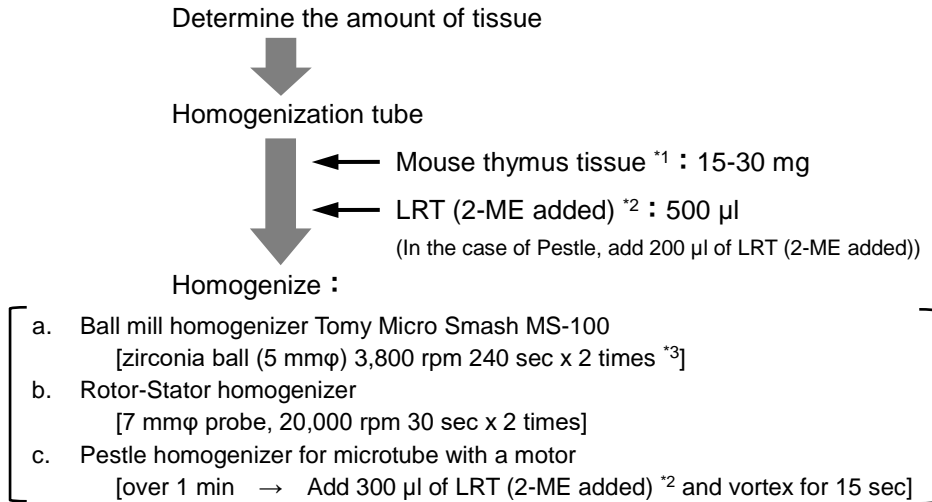
Mouse Liver, Mouse Brain, Mouse Lung, Mouse Kidney, Mouse Spleen

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).

RA-b-21

Total RNA Extraction from Thymus of Mouse

Protocol 1 (15-30 mg)



*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

*3 In the case of Thymus, TOMY Micro Smash MS-100R (with a cooler) may yield more compared with MS-100.

17,000 x g (15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

← SRT : 175 µl

Vortex (maximum speed) : 15 sec

Flash spin down

← >99% ethanol : 140 µl

Vortex (maximum speed) : 1 min

Flash spin down

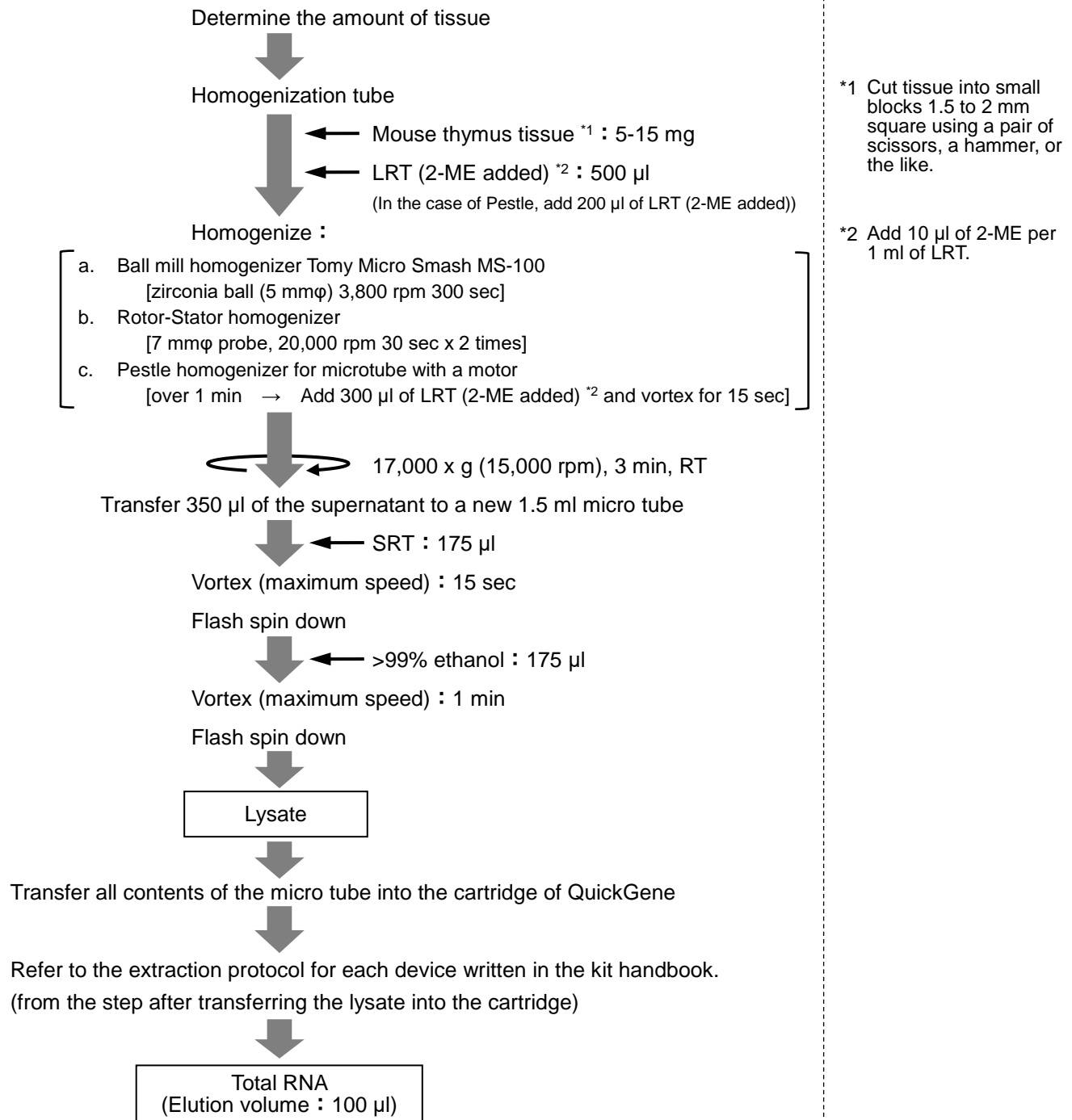
Lysate

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 : 100 µl)

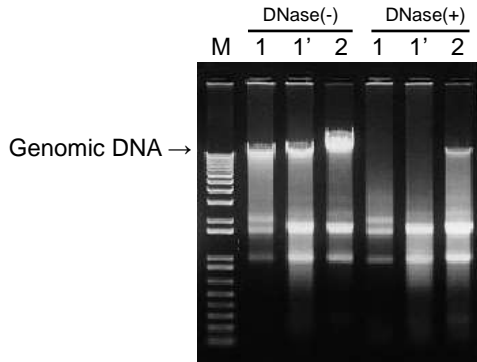
Protocol 2 (5-15 mg)



Results

Electropherogram

Nondenaturing Agarose gel electrophoresis was performed with total RNA.
Electrophoresis conditions : 1% Agarose / 1 x TAE



M : Marker (1 kb PLUS DNA Ladder : Invitrogen)
1 : QuickGene (with MS-100)
1' : QuickGene (with MS-100R (with a cooler))
2 : Competitor A kit (spin column method, for Fibrous)

For thymus etc., QuickGene system enables extraction of total RNA with genomic DNA contamination less than that in the case of Competitor A kit (spin column method).

The yield of total RNA

Tissue	Ball mill homogenizer (MS-100)			Rotor-Stator homogenizer		
	Tissue amount	DNase(+)	DNase(-)	Tissue amount	DNase(+)	DNase(-)
Thymus	30 mg	43 µg	27 µg	5 mg	19 µg	17 µg

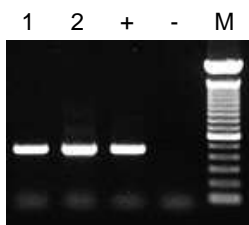
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Thymus	30 mg	2.17	2.17	2.15	2.17

Other

RT-PCR

RT-PCR was performed on total RNA.



< RT reaction conditions >

Template : Total RNA from mouse thymus (with DNase treatment) 500 ng
Enzyme : SuperScript II (Invitrogen)

< PCR conditions >

Template : cDNA equivalent to total RNA (10 pg/µl)
Primer : *G3PDH* primer
Enzyme : Takara Taq Hot Start Version (TaKaRa)

< Electrophoresis condition >

1% Agarose / 1 x TAE
M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Competitor A kit (spin column method)
+ : Positive control (mLiver RNA : Clontech)
- : Negative control (RNase-free water)

Common protocol is usable for the following

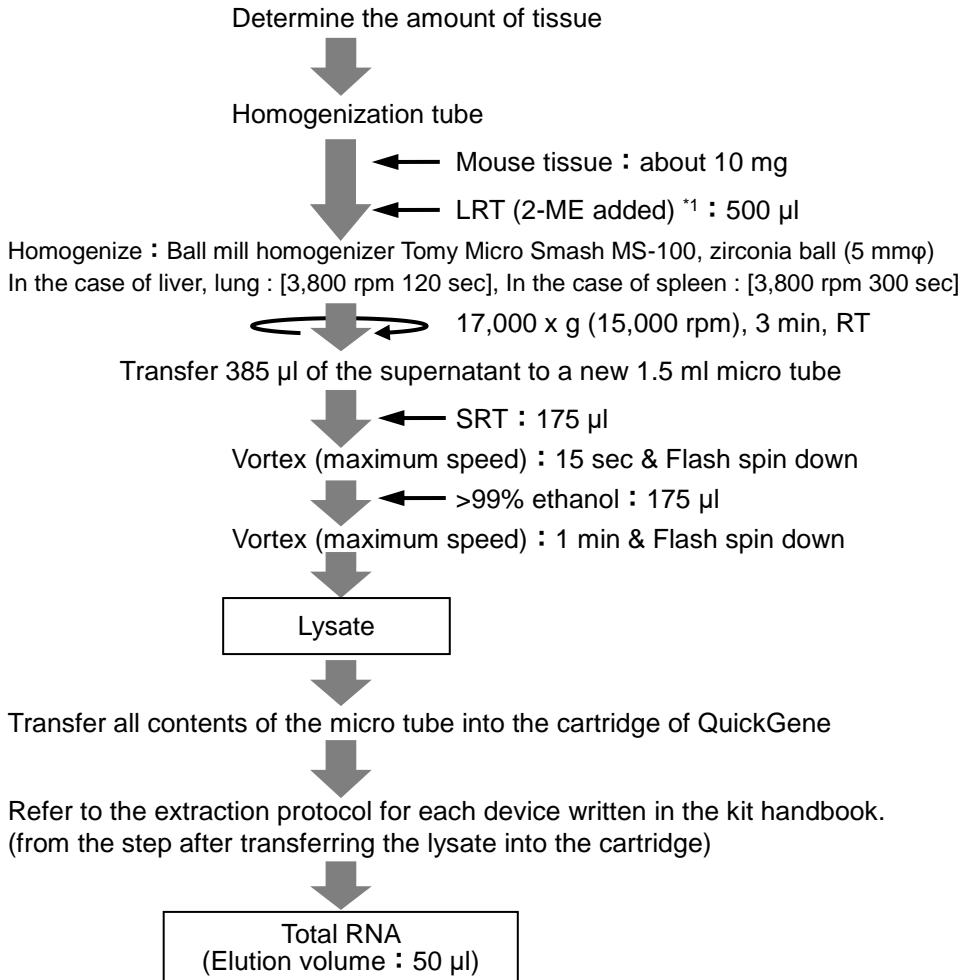
No Data

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).

RA-b-22

Total RNA Extraction from Mouse Tissue for DNA chip "Genopal®"

Protocol



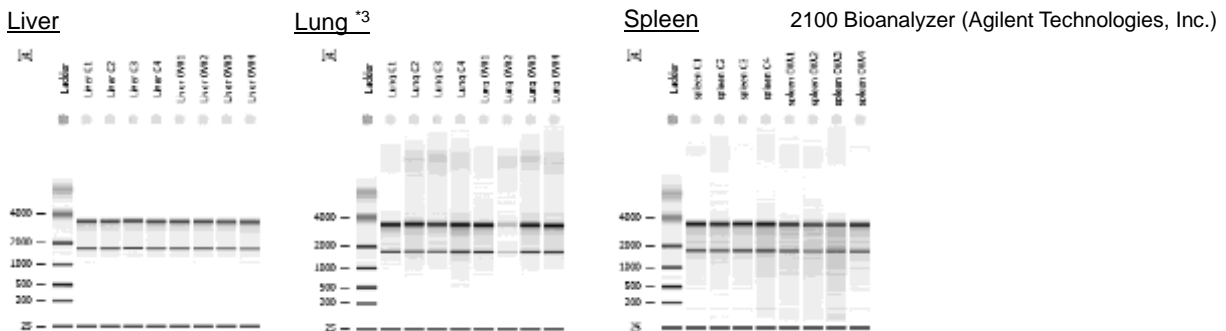
*1 Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, or the like.

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Electropherogram

Electrophoresis was performed with total RNA extracted from various tissue of mouse using QuickGene system (with Ball mill homogenizer).



*3 The result obtained by two concentrated samples. Two samples were separately extracted then combined before concentrated.

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).

The yield of total RNA

Tissue	Yield (μg)							
	C1	C2	C3	C4	OVA1	OVA2	OVA3	OVA4
Liver	65.9	56.2	59.5	72.2	63.0	50.6	69.7	96.1
Lung *3	10.6	5.1	4.9	8.1	9.3	2.5	6.2	6.2
Spleen	33.2	23.6	40.8	30.0	27.6	24.5	32.2	47.4

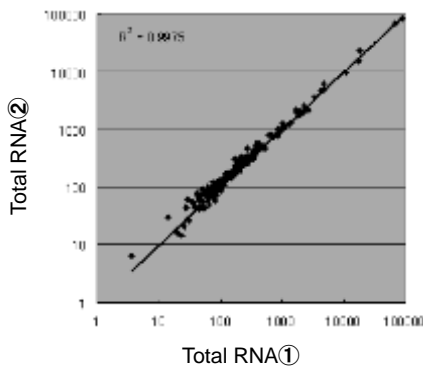
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Tissue	Tissue amount	A260/280		A260/230	
		DNase(+)	DNase(-)	DNase(+)	DNase(-)
Thymus	30 mg	2.17	2.17	2.15	2.17

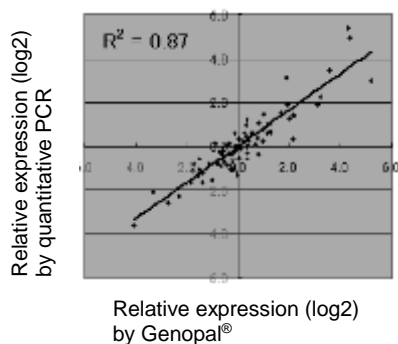
Other

Genopal® Analysis

Fluorescent intensity of each gene of the sample was measured according to standard protocol of Allergy chip "Genopal®" (ARIM-GX, Mitsubishi Rayon Co., Ltd.) arrayed with 209 probes corresponding to mouse genes, and relative expression (log2 ratio) between each group was calculated.



Data obtained with aRNA specimen prepared from total RNA extracted independently of the same sample demonstrated high reproducibility.



The numeric character data of the relative expression that had been obtained by Allergy chip "Genopal®" and quantitative PCR showed high correlation (R2=0.87).

Common protocol is usable for the following

No Data

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

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