17. Total RNA Extraction from Cultured Cell

RG-1

Total RNA Extraction Cultured COS-7 Cells (For ~ 1 x 10^6 cells)

Protocol

Pelleted cells
Pellet cells and remove all supernatant by aspiration
(Do not use more than 1×10^6 cells)

← PBS: 20 μl (For frozen cell pellet)

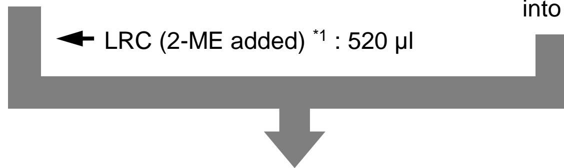
Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1 : 520 μl

On-dish lysis
Remove all medium in the dish by aspiration
(Do not use more than 1×10^6 cells)

← LRC (2-ME added) *1 : 520 μl

Collect lysed cells with a cell scraper into a micro tube



Homogenization Protocol I (Vortex method) :
- Mix thoroughly by vortexing for 1 min with maximum speed

Homogenization Protocol II (Needle and syringe method) :
- Pass the lysate 15 times through a 21-gauge needle fitted to 1 ml or 2.5 ml syringe. *2 *3

Homogenization Protocol III (Rotor-Stator homogenizer method) :
- Transfer cell lysate with a pipette into a container for homogenization
  (Round bottom 2 ml plastic tube is recommended.) *4

Flash spin down

← >99% ethanol : 100 μl

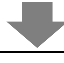
Vortex (maximum speed) : 15 sec

Flash spin down

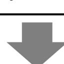
← >99% ethanol : 180 μl

Vortex (maximum speed) : 15 sec

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)

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 μl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

*4 Homogenize Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmφ or 7 mmφ generator is used

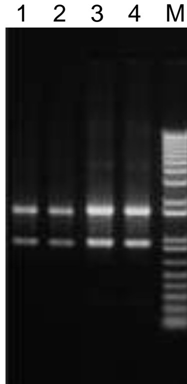
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram

COS-7 (1 well / 6 well Plate (3.5 cm dish plate), 6 cm dish)



1,2 : 1 well / 6 well Plate (3.5 cm dish plate), Homogenization protocol II
3,4 : 6 cm dish, Homogenization protocol III
M : Ready Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Sample	Number of cells	Homogenization protocol	Yield (µg)	A260/280	A260/230
COS-7	0.3×10^6	II	13.6	2.19	2.19
	0.8×10^6	III	34.4	1.96	2.17

Common protocol is usable for the following

Cultured HeLa Cells, Cultured HEK293 Cells, Cultured NIH/3T3 Cells

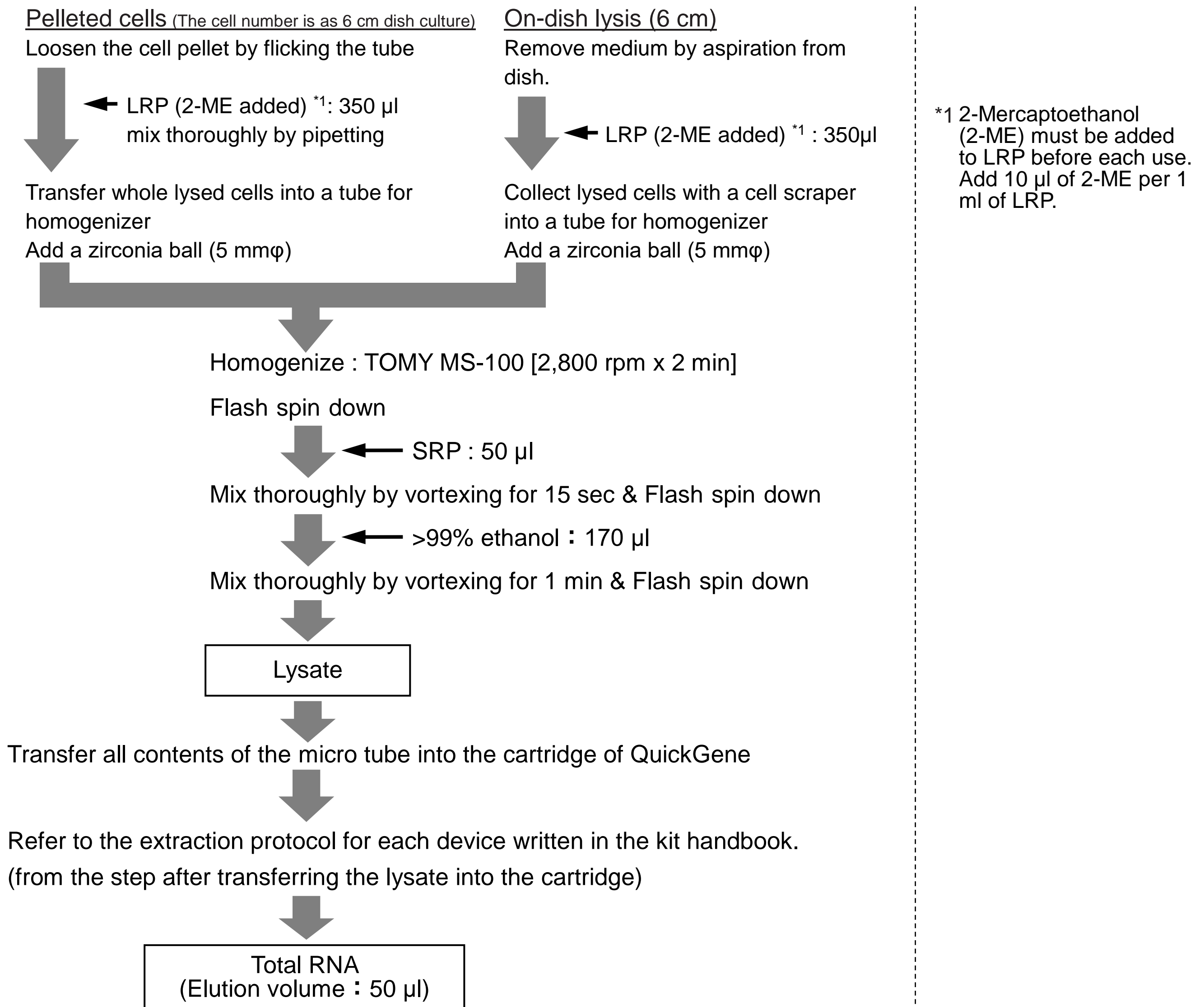
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

RG-2

Total RNA Extraction from Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl of 2-ME per 1 ml of LRP.

Results

Lysing adherent cells directly in 6 cm dish, or lysing pelleted floating cells of 6 cm dish, total RNA was isolated.

The yield of total RNA (with DNase treatment)

Cell Line	Number of cells (x 10^6 cells)	Yield (µg)
COS-7	1.0	42.3

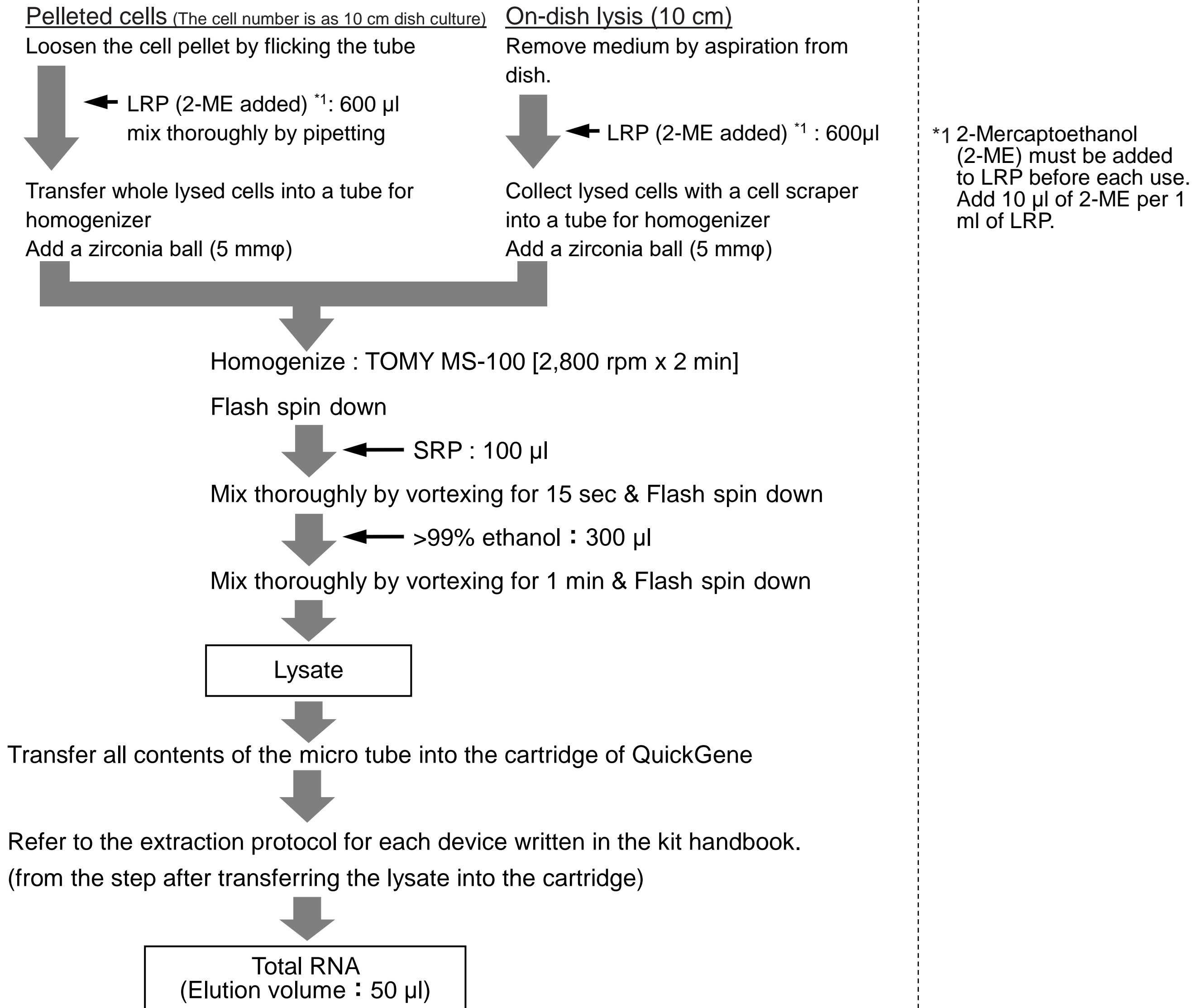
Common protocol is usable for the following

Cultured HeLa Cells, Cultured HEK293 Cells, NIH/3T3 Cells

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).

KURABO

Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl of 2-ME per 1 ml of LRP.

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).

Results

Lysing adherent cells directly in 10 cm dish, or lysing pelleted floating cells of 10 cm dish, total RNA was isolated.

The yield of total RNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

Sample	Number of cells	Protocol	DNase	Yield (μg)	A260/280	A260/230
COS-7	2.5×10^6	QuickGene	+	104.2	2.12	2.11
			-	90.0	2.12	1.94
		Spin column method (A Company)	+	98.2	1.97	2.03
			-	79.0	2.05	2.19

By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Common protocol is usable for the following

Cultured HeLa Cells, Cultured HEK293 Cells, NIH/3T3 Cells

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).

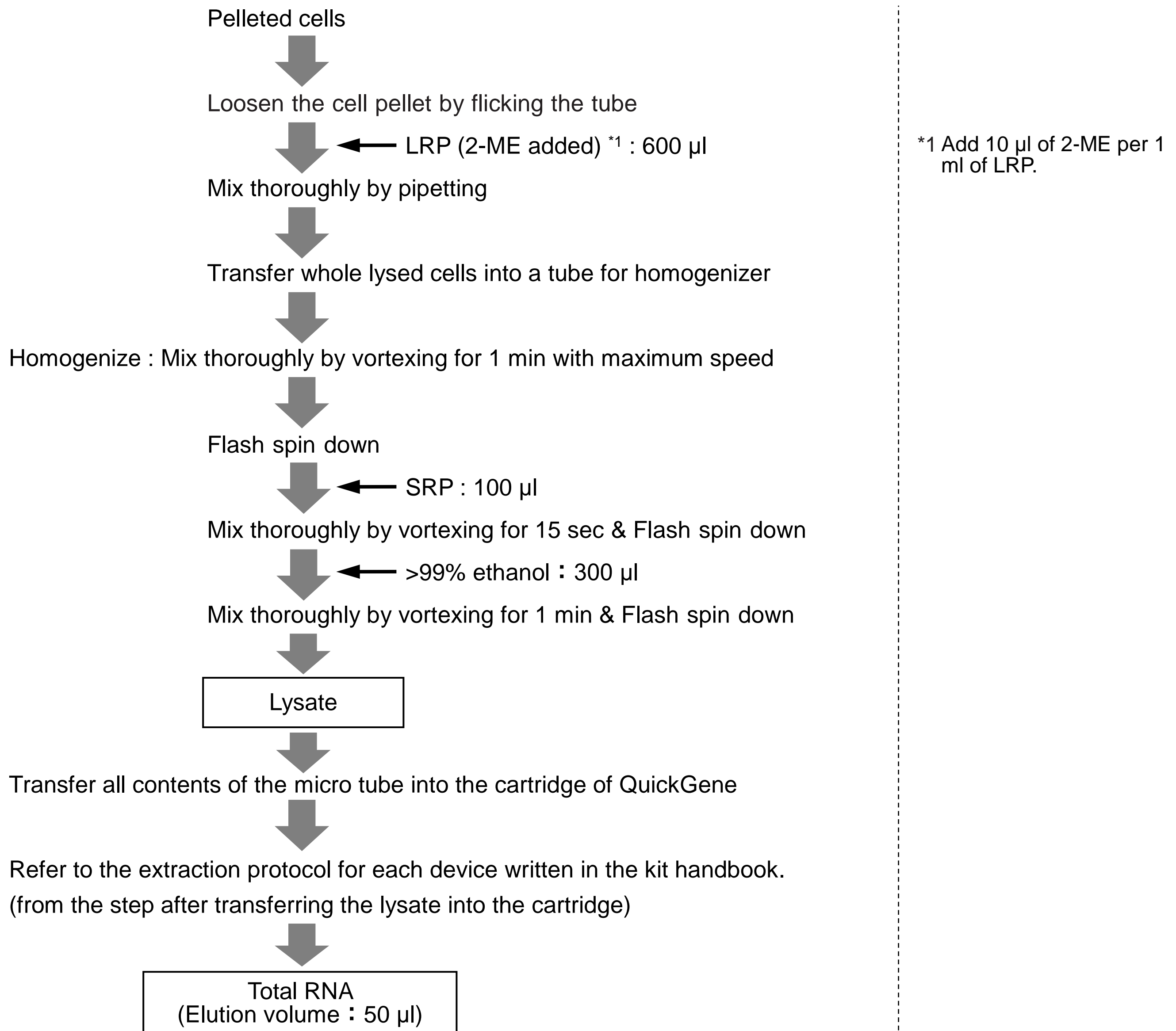
KURABO

QuickGene

RG-3

Total RNA Extraction from Cultured ES Cells

Protocol



Results

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

Number of lymphocytes	Yield (µg)	A260/280
2 x 10^6 cells	41.4 (2 cartridges)	2.1

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).

KURABO

RG-4

Total RNA Extraction from Cultured HEK293 Cells (For ~ 1 x 10^6 cells)

Protocol

Pelleted cells
Pellet cells and remove all supernatant by aspiration
(Do not use more than 1×10^6 cells)

← PBS: 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1 : 520 µl

On-dish lysis
Remove all medium in the dish by aspiration
(Do not use more than 1×10^6 cells)

← LRC (2-ME added) *1 : 520 µl

Collect lysed cells with a cell scraper into a micro tube

Homogenization Protocol I (Vortex method) :
- Mix thoroughly by vortexing for 1 min with maximum speed

Homogenization Protocol II (Needle and syringe method) :
- Pass the lysate 15 times through a 21-gauge needle fitted to 1 ml or 2.5 ml syringe. *2 *3

Homogenization Protocol III (Rotor-Stator homogenizer method) :
- Transfer cell lysate with a pipette into a container for homogenization
  (Round bottom 2 ml plastic tube is recommended.) *4

Flash spin down

← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec

Flash spin down

← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec

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)

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

*4 Homogenize Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmφ or 7 mmφ generator is used

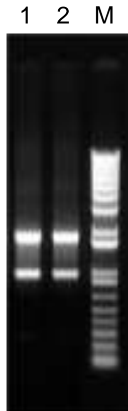
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram

HEK293 (1 well / 6 well Plate (3.5 cm dish plate))



1,2 : Homogenization protocol II
M : Ready-Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Sample	Number of cells	Homogenization protocol	Yield (μg)	A260/280	A260/230
HEK293	2.1×10^6	II	30.4	2.27	2.14

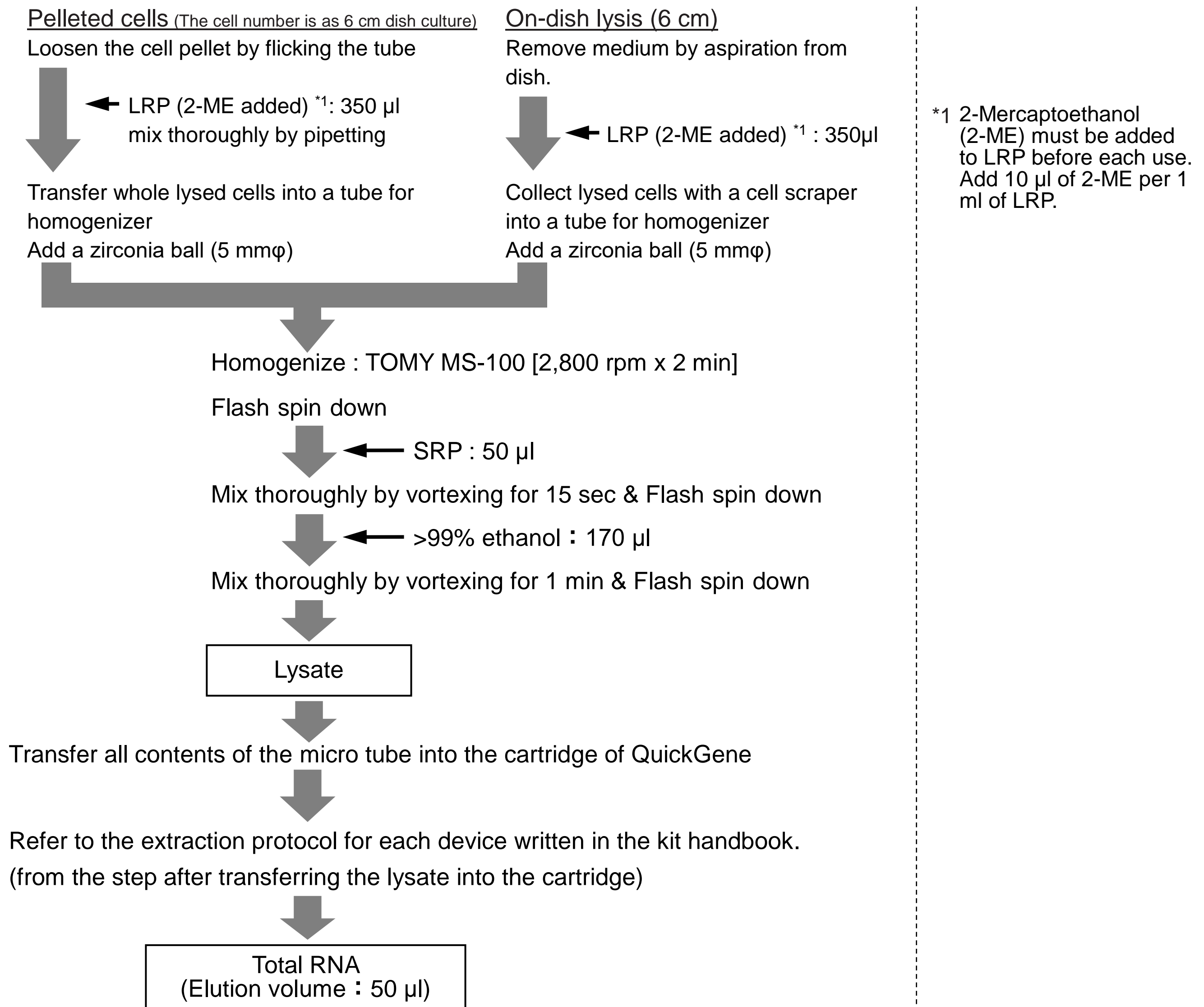
Common protocol is usable for the following

Cultured COS-7 Cells, Cultured HeLa Cells, Cultured NIH/3T3 Cells

RG-5

Total RNA Extraction from Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl of 2-ME per 1 ml of LRP.

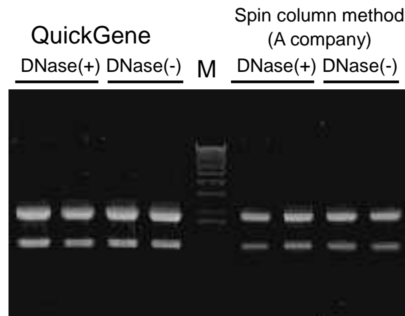
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Lysing adherent cells directly in 6 cm dish, or lysing pelleted floating cells of 6 cm dish, total RNA was isolated.

Electropherogram



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

The yield of total RNA (with DNase treatment)

Sample	Number of cells (x 10^6 cells)	Protocol	Yield (μg)
HEK293	5.0	QuickGene	79.1
		Spin column method (A company)	57.5

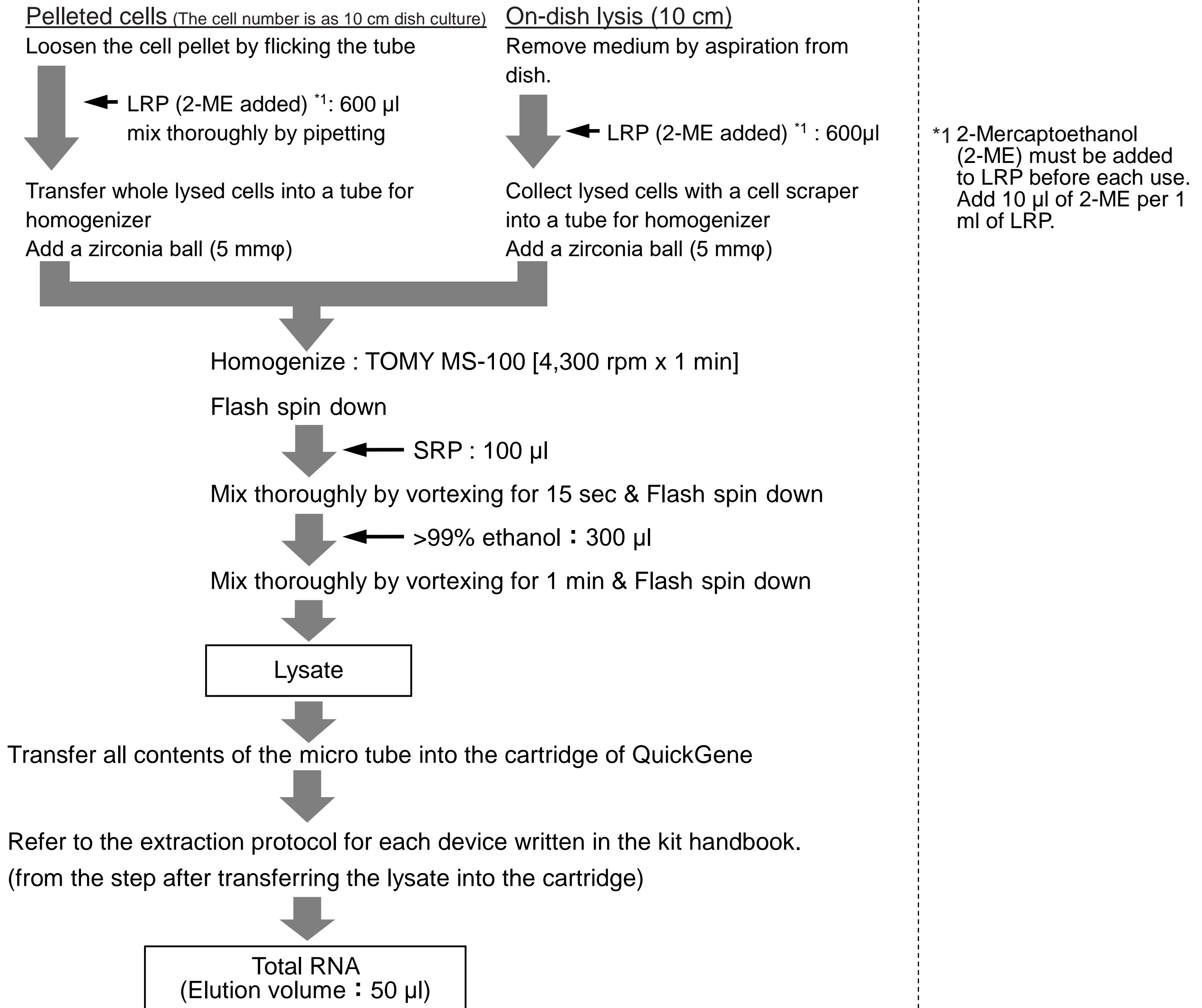
Common protocol is usable for the following

Cultured HeLa Cells, Cultured COS-7 Cells, NIH/3T3 Cells

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).

KURABO

Protocol B



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).

Results

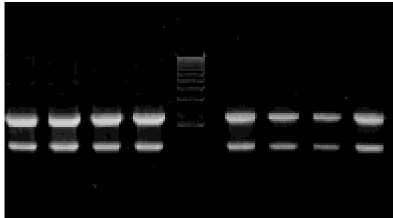
Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HEK293 (10cm dish)

QuickGene DNase(+) DNase(-) M Spin column method (A company) DNase(+) DNase(-)



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

The yield of total RNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

Sample	Number of cells	Protocol	DNase	Yield (μg)	A260/280	A260/230
HEK293	5.0 - 8.0 x 10^6	QuickGene	+	175.3	2.29	2.12
			-	160.3	2.27	2.11
		Spin column method (A Company)	+	92.2	2.11	2.16
			-	101.0	2.11	2.18

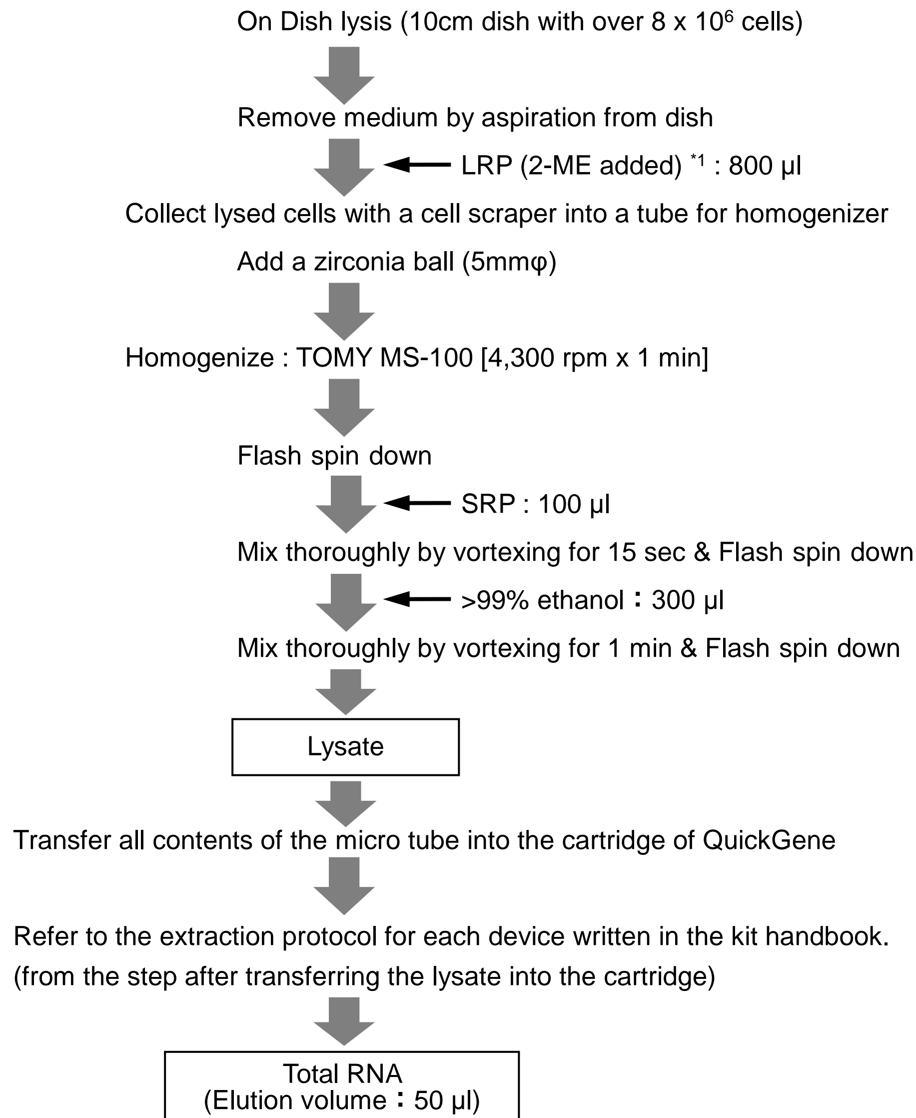
By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Common protocol is usable for the following

Cultured HeLa Cells, Cultured COS-7 Cells, NIH/3T3 Cells

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).

Protocol B'



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl of 2-ME per 1 ml of LRP.

Results

Total RNA was isolated from cultured cells, HEK293, using QuickGene system (QuickGene and QuickGene RNA cultured cell HC kit S) and Spin column method (A company).

The yield of total RNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

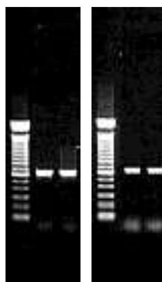
Sample	Number of cells	Protocol	DNase	Yield (μg)	A260/280	A260/230
HEK293	12 x 10^6	QuickGene	+	149.5	1.95	2.14
			-	94.9	1.98	1.88
		Spin column method (A Company)	+	133.1	2.04	2.14
			-	102.3	2.02	2.17

By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

▪ RT-PCR (with DNase treatment)

RT-PCR was performed with *ß-actin* mRNA as the template on total RNA (10 pg/μl or 1 pg/μl) isolated using QuickGene system and Spin column method (A company).



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)

For RT-PCR performed on total RNA (1 pg/μl), similar electrophoretic bands of the amplification products were detected for both kits.

Common protocol is usable for the following

Cultured HeLa Cells, Cultured COS-7 Cells, NIH/3T3 Cells

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).
KURABO

RG-6

Total RNA Extraction from Cultured HeLa Cells (For ~ 1 x 10^6 cells)

Protocol

Pelleted cells
Pellet cells and remove all supernatant by aspiration
(Do not use more than 1 x 10^6 cells)

← PBS: 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1 : 520 µl

On-dish lysis
Remove all medium in the dish by aspiration
(Do not use more than 1 x 10^6 cells)

← LRC (2-ME added) *1 : 520 µl

Collect lysed cells with a cell scraper into a micro tube

Homogenization Protocol I (Vortex method) :
- Mix thoroughly by vortexing for 1 min with maximum speed

Homogenization Protocol II (Needle and syringe method) :
- Pass the lysate 15 times through a 21-gauge needle fitted to 1 ml or 2.5 ml syringe. *2 *3

Homogenization Protocol III (Rotor-Stator homogenizer method) :
- Transfer cell lysate with a pipette into a container for homogenization
  (Round bottom 2 ml plastic tube is recommended.) *4

Flash spin down

← >99% ethanol：100 µl

Vortex (maximum speed) : 15 sec

Flash spin down

← >99% ethanol：180 µl

Vortex (maximum speed) : 15 sec

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)

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

*4 Homogenize Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmφ or 7 mmφ generator is used

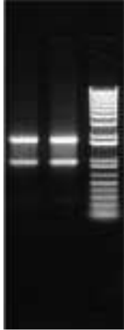
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

HeLa (1 well / 6 well Plate (3.5 cm dish plate))



1,2 : Homogenization protocol II
M : Ready-Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Sample	Number of cells	Homogenization protocol	Yield (µg)	A260/280	A260/230
HeLa	1.2×10^6	II	28.1	2.28	2.21

Common protocol is usable for the following

Cultured COS-7 Cells, Cultured HEK293 Cells, Cultured NIH/3T3 Cells

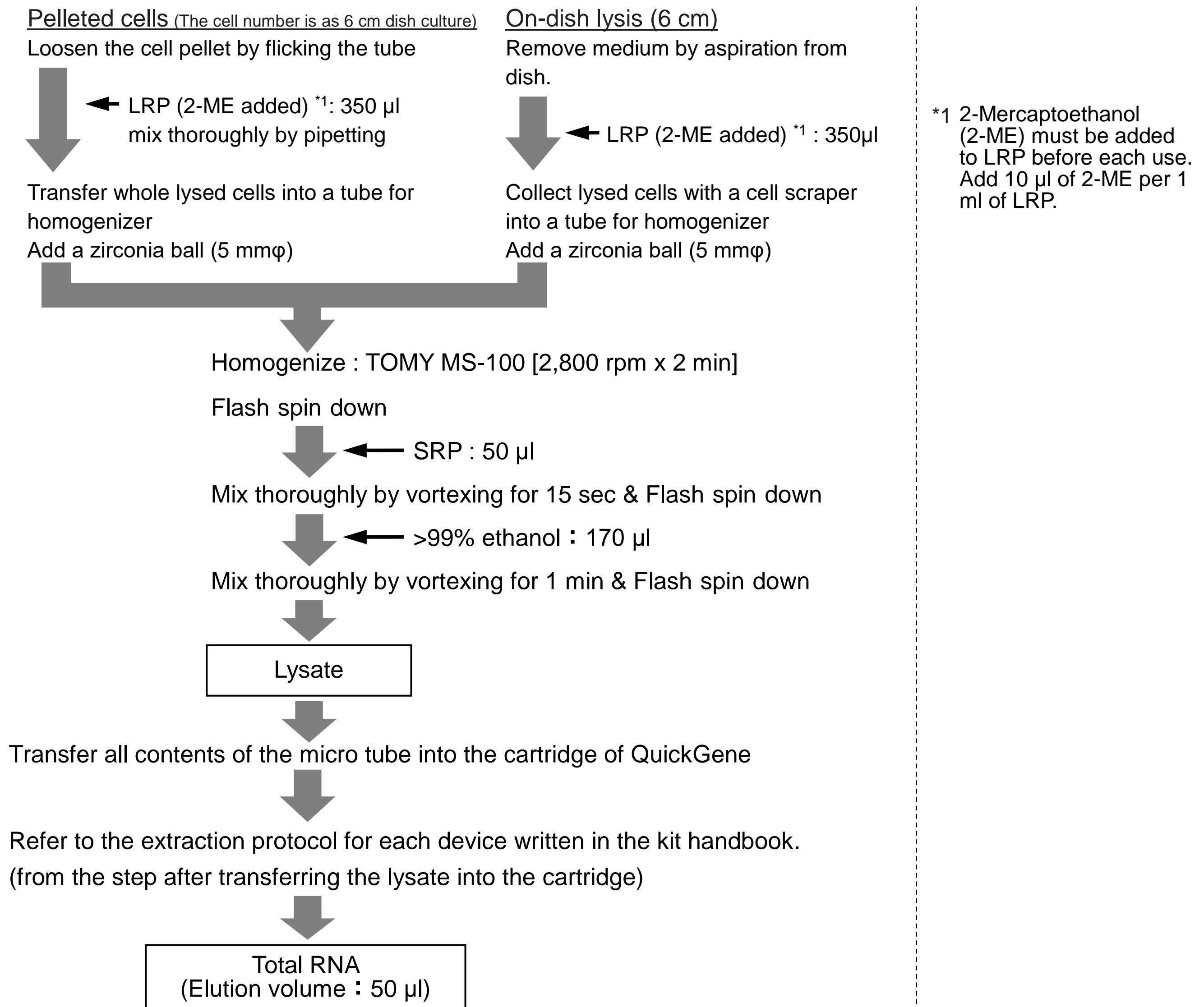
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

RG-7

Total RNA Extraction from Cultured HeLa Cells (For cells cultured in 6 cm or 10 cm dish)

Protocol A



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).

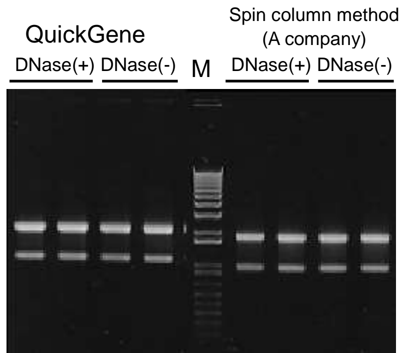
Results

Lysing adherent cells directly in 6 cm dish, or lysing pelleted floating cells of 6 cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HeLa (2 x 10^6 cells)



M : Marker (1Kb Plus DNA Ladder : Invitrogen)

The yield of total RNA (with DNase treatment)

Sample	Number of cells (x 10^6 cells)	Protocol	Yield (µg)
HEK293	2.0	QuickGene	47.2
		Spin column method (A company)	46.1

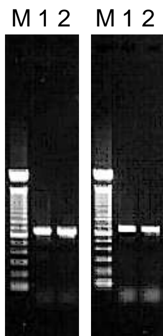
Other

- RT-PCR (with DNase treatment)

RT-PCR was performed with *ß-actin* mRNA as the template on total RNA (10 pg/µl or 1 pg/µl) isolated using QuickGene system and Spin column method (A company).

HeLa (6 cm dish)

10 pg/µl 1 pg/µl



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)

Common protocol is usable for the following

Cultured HEK293 Cells, Cultured COS-7 Cells, NIH/3T3 Cells

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).

Protocol B

Pelleted cells (The cell number is as 10 cm dish culture)

Loosen the cell pellet by flicking the tube

← LRP (2-ME added) *1: 600 μl
mix thoroughly by pipetting

Transfer whole lysed cells into a tube for homogenizer
Add a zirconia ball (5 mmφ)

On-dish lysis (10 cm)

Remove medium by aspiration from dish.

← LRP (2-ME added) *1 : 600μl

Collect lysed cells with a cell scraper into a tube for homogenizer
Add a zirconia ball (5 mmφ)

Homogenize : TOMY MS-100 [4,300 rpm x 1 min]

Flash spin down

← SRP : 100 μl

Mix thoroughly by vortexing for 15 sec & Flash spin down

← >99% ethanol : 300 μl

Mix thoroughly by vortexing for 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 : 50 μl)

*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 μl of 2-ME per 1 ml of LRP.

Results

Lysing adherent cells directly in 10 cm dish, or lysing pelleted floating cells of 10 cm dish, total RNA was isolated.

Electropherogram

Nondenaturing Gel Electrophoresis (1% Agarose / 1 x TAE Buffer)

HeLa (10cm dish)

QuickGene		M	Spin column method (A company)	
DNase(+)	DNase(-)		DNase(+)	DNase(-)

M : Marker (1Kb Plus DNA Ladder : Invitrogen)

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).

KURABO

The yield of total RNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

Sample	Number of cells	Protocol	DNase	Yield (μg)	A260/280	A260/230
HeLa	5.0 x 10^6	QuickGene	+	129.0	2.20	2.18
			-	122.0	2.20	2.05
		Spin column method (A Company)	+	115.7	1.99	2.10
			-	104.0	2.02	2.12

Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

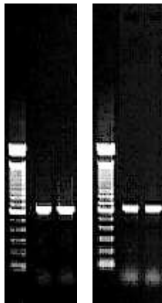
- RT-PCR (with DNase treatment)

RT-PCR was performed with *ß-actin* mRNA as the template on total RNA (10 pg/μl or 1 pg/μl) isolated using QuickGene system and Spin column method (A company).

HeLa (10 cm dish)

10 pg/μl 1 pg/μl

M 1 2 M 1 2



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)

Common protocol is usable for the following

Cultured HEK293 Cells, Cultured COS-7 Cells, NIH/3T3 Cells

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).

RG-8

Total RNA Extraction from Cultured HL60 Cells (For ~ 1 x 10^6 cells)

Protocol

Pellet cells and remove all supernatant by aspiration
(Do not use more than 1 x 10^6 cells)

↓ ← PBS : 20 µl (For frozen cell pellet)

Loosen the cell pellet by flicking the tube

↓ ← LRC (2-ME added) *1 : 520 µl

Vortex (maximum speed) : 1 min & Flash spin down

↓ ← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓ ← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

The yield of total RNA / Protein contamination : A260/280 / Chaotropic salt contamination : A260/230

Sample	Number of cells	Yield (µg)	A260/280	A260/230
HL60	1.0 x 10^6	9.7	1.88	2.08

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).

RG-9

Total RNA Extraction from Cultured Lens Epithelial Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

↓

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

↓ ← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓ ← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

Protein contamination : A260/280

Sample	Number of cells	A260/280
Lens Epithelial Cells	1.0 x 10^6	1.77

Common protocol is usable for the following

Cultured MCF-7 Cells, HuH-7 Cells, Cultured Smooth muscle Cells, Cultured PC12 Cells, Cultured Porcine fat Cells, Cultured Periodontal ligament Cells

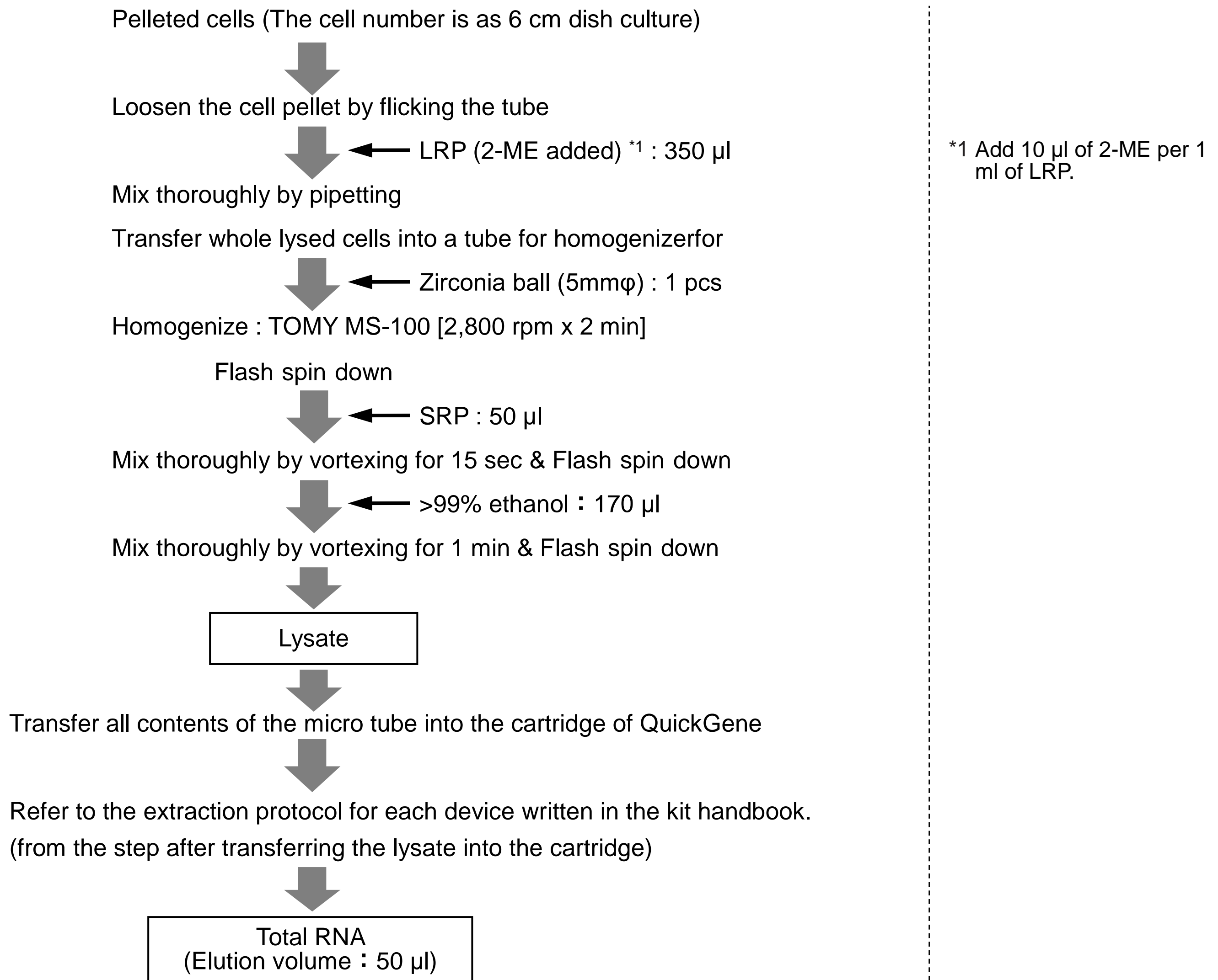
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

RG-10

Total RNA Extraction from Cultured Lymphocytes

Protocol



Results

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

Sample	Number of cells	Yield (µg)	A260/280
Lymphocytes	1.0×10^6	13.4	1.67

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).

KURABO

RG-11

Total RNA Extraction from Cultured NIH/3T3 Cells (For ~ 1 x 10^6 cells)

Protocol

Pelleted cells
Pellet cells and remove all supernatant by aspiration
(Do not use more than 1 x 10^6 cells)

← PBS: 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1 : 520 µl

On-dish lysis
Remove all medium in the dish by aspiration
(Do not use more than 1 x 10^6 cells)

← LRC (2-ME added) *1 : 520 µl

Collect lysed cells with a cell scraper into a micro tube

↓

Homogenization Protocol I (Vortex method) :
- Mix thoroughly by vortexing for 1 min with maximum speed

Homogenization Protocol II (Needle and syringe method) :
- Pass the lysate 15 times through a 21-gauge needle fitted to 1 ml or 2.5 ml syringe. *2 *3

Homogenization Protocol III (Rotor-Stator homogenizer method) :
- Transfer cell lysate with a pipette into a container for homogenization
  (Round bottom 2 ml plastic tube is recommended.) *4

Flash spin down

← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec

Flash spin down

← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec

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)

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl of 2-ME per 1 ml of LRC.

*2 In order to prevent foaming of a sample, please avoid mixing of air.

*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

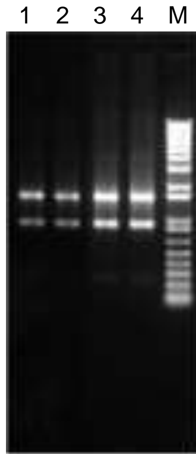
*4 Homogenize Condition example : 20,000 rpm, for 30 sec, 2 times
5 mmφ or 7 mmφ generator is used

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).

Results

Electropherogram

NIH/3T3 (1 well / 6 well Plate (3.5 cm dish plate), 6 cm dish)



1,2 : 1 well / 6 well Plate (3.5 cm dish plate), Homogenization protocol I
3,4 : 6 cm dish, Homogenization protocol II
M : Ready-Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)
Protein contamination : A260/280 /Chaotropic salt contamination : A260/230

Sample	Number of cells	Homogenization protocol	Yield (µg)	A260/280	A260/230
NIH/3T3	0.3×10^6	I	15.6	2.17	2.18
	1.2×10^6	II	22.6	2.26	2.22

Common protocol is usable for the following

Cultured COS-7 Cells, Cultured HeLa Cells, Cultured HEK293 Cells

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).

KURABO

RG-12

Total RNA Extraction from Cultured Periodontal Ligament Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

↓

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

↓ ← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓ ← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

The yield of total RNA / Protein contamination : A260/280 / Chaotropic salt contamination : A260/230

Sample	Number of cells	Yield (µg)	A260/280	A260/230
Periodontal Ligament Cells	1.0 x 10^5	1.2	1.9	1.2

Common protocol is usable for the following

Cultured MCF-7 Cells, HuH-7 Cells, Cultured Smooth muscle Cells, Cultured PC12 Cells, Cultured Porcine fat Cells, Cultured Lens epithelial Cells

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).

KURABO

RG-13

Total RNA Extraction from Cultured Porcine Fat Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

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

Sample	Kind of cells	Yield (µg)	A260/280
Porcine Fat Cells	differentiated cells	0.6	2.09
	undifferentiated cells	1.2	2.07

Common protocol is usable for the following

Cultured MCF-7 Cells, HuH-7 Cells, Cultured Smooth muscle Cells, Cultured PC12 Cells, Cultured Lens epithelial Cells, Cultured Periodontal ligament Cells

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).

KURABO

Total RNA Extraction from HL60 Cells (For cells cultured in 6 cm or 10 cm dish)

Protocol A

Pelleted cells (The cell number is as 6 cm dish culture)

Loosen the cell pellet by flicking the tube

← LRP (2-ME added) [*1] : 350 µl

Mix thoroughly by pipetting

Transfer whole lysed cells into a tube for homogenizerfor

← Zirconia ball (5mmφ) : 1 pcs

Homogenize : TOMY MS-100 [2,800 rpm x 2 min]

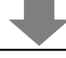
Flash spin down

← SRP : 50 µl

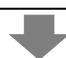
Mix thoroughly by vortexing for 15 sec & Flash spin down

← >99% ethanol：170 µl

Mix thoroughly by vortexing for 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：50 µl)

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl of 2-ME per 1 ml of LRC.

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).

KURABO

Results

Lysing adherent cells directly in 6 cm dish, or lysing pelleted floating cells of 6 cm dish, total RNA was isolated.

The yield of total RNA (with DNase treatment)

Sample	Number of cells (x 10^6 cells)	Protocol	Yield (μg)
HL60	5.0	QuickGene	33.1
		Spin column method (A company)	46.2

Other

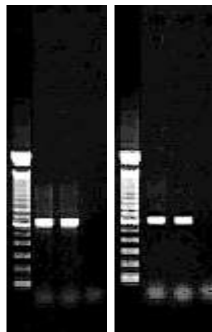
▪ RT-PCR (with DNase treatment)

RT-PCR was performed with *ß-actin* mRNA as the template on total RNA (10 pg/μl or 1 pg/μl) isolated using QuickGene system and Spin column method (A company).

HeLa (6 cm dish)

10 pg/μl 1 pg/μl

M 1 2 N M 1 2 N



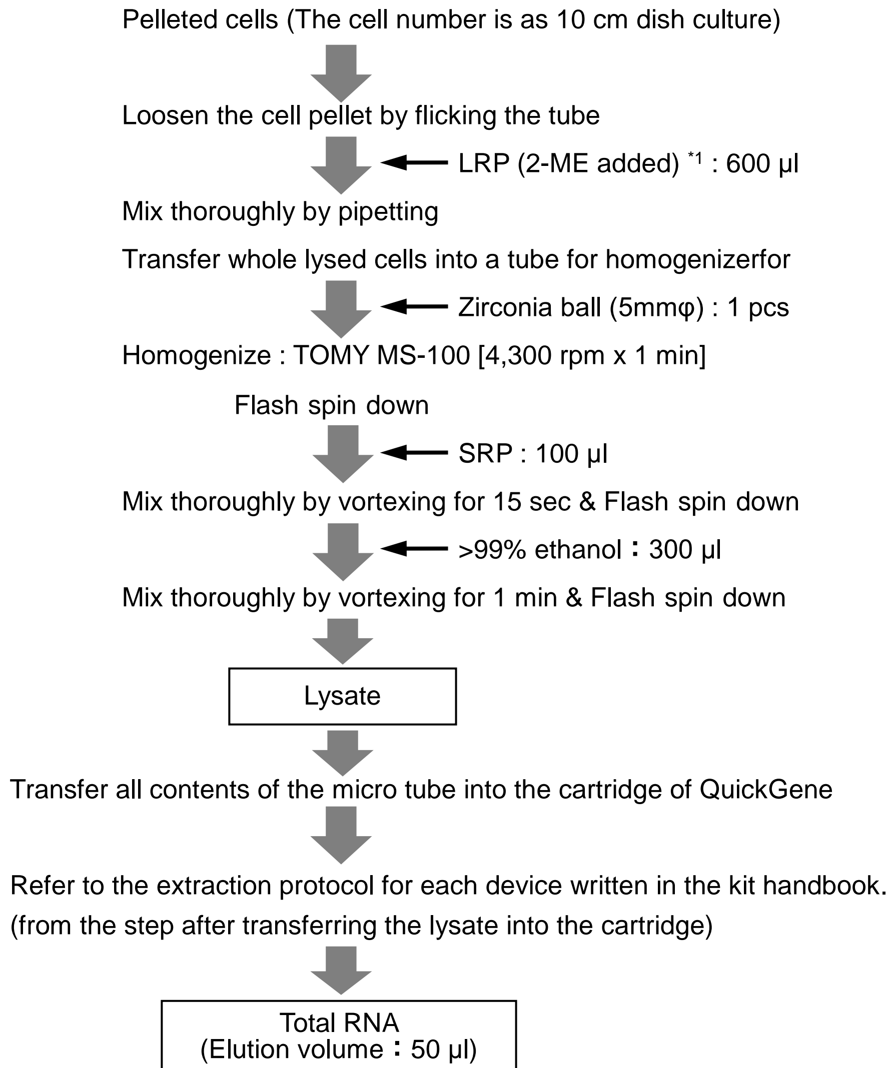
M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

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).

Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl of 2-ME per 1 ml of LRC.

Results

Lysing adherent cells directly in 10 cm dish, or lysing pelleted floating cells of 10 cm dish, total RNA was isolated.

The yield of total RNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

Sample	Number of cells	Protocol	DNase	Yield (μg)	A260/280	A260/230
HL60	15.0×10^6	QuickGene	+	167.3	1.92	2.17
			-	144.4	2.18	2.18
		Spin column method (A Company)	+	154.4	1.85	2.15
			-	140.5	2.09	2.12

By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

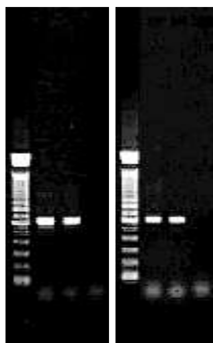
▪ RT-PCR

RT-PCR was performed with *ß-actin* mRNA as the template on total RNA (10 pg/μl or 1 pg/μl) isolated using QuickGene system and Spin column method (A company).

HL60 (15.0×10^6 cells)

10 pg/μl 1 pg/μl

M 1 2 N M 1 2 N



M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

For RT-PCR performed on total RNA (1 pg/μl), similar electrophoretic bands of the amplification products were detected for both kits.

Common protocol is usable for the following

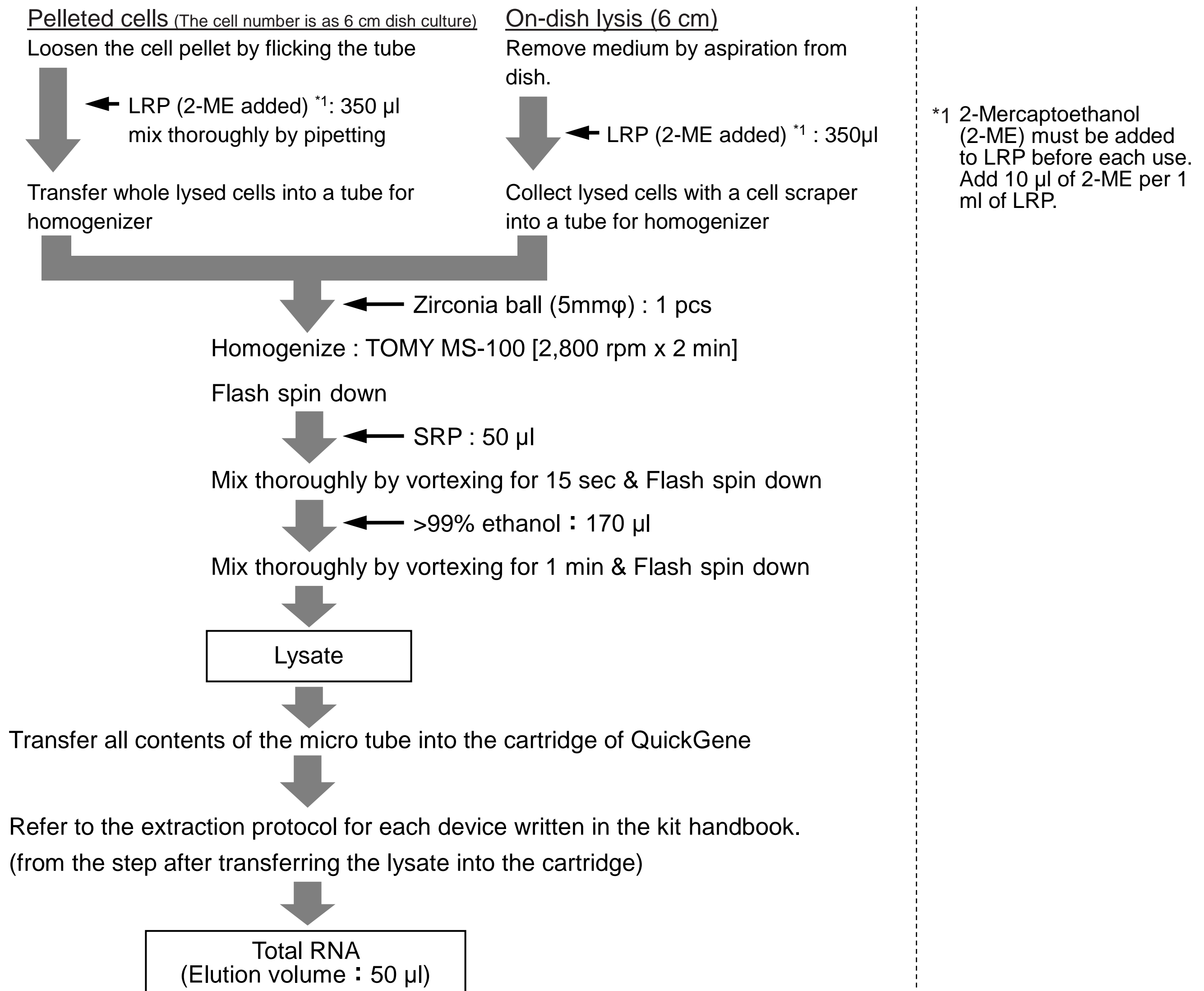
No Data

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).

RG-15

Total RNA Extraction from NIH/3T3 Cells (For cells cultured in 6 cm or 10 cm dish)

Protocol A



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl of 2-ME per 1 ml of LRP.

Results

Lysing adherent cells directly in 6 cm dish, or lysing pelleted floating cells of 6 cm dish, total RNA was isolated.

The yield of total RNA (with DNase treatment)

Sample	Number of cells (x 10^6 cells)	Protocol	Yield (µg)
NIH/3T3	1.5	QuickGene	27.9
		Spin column method (A company)	35.7

Common protocol is usable for the following

Cultured HeLa Cells, Cultured HEK293 Cells, Cultured COS-7 Cells

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).

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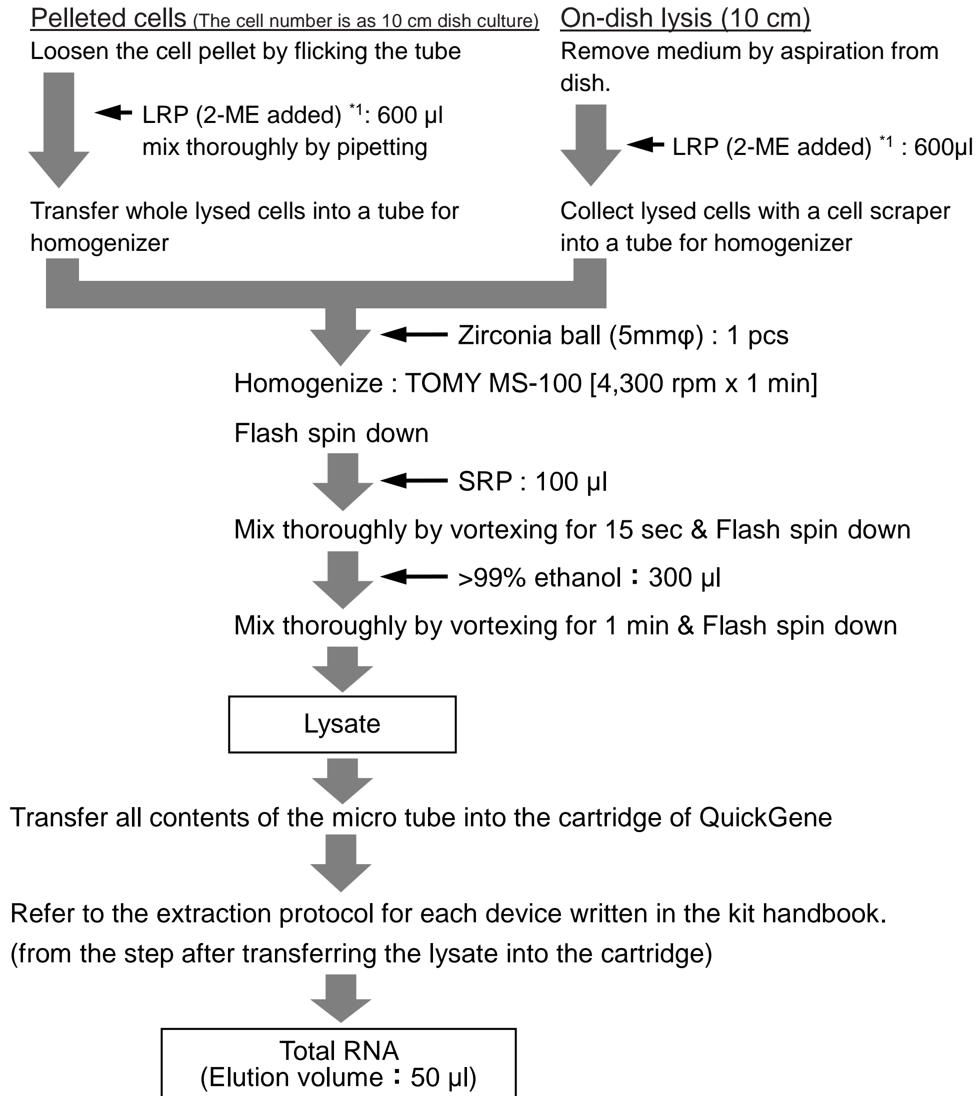
Protocol B



*1 2-Mercaptoethanol (2-ME) must be added to LRP before each use. Add 10 µl of 2-ME per 1 ml of LRP.

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).

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Results

Lysing adherent cells directly in 10 cm dish, or lysing pelleted floating cells of 10 cm dish, total RNA was isolated.

The yield of total RNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

Sample	Number of cells	Protocol	DNase	Yield (µg)	A260/280	A260/230
NIH/3T3	4.5×10^6	QuickGene	+	89.4	2.19	2.02
			-	79.0	2.17	1.94
		Spin column method (A Company)	+	100.2	2.02	2.26
			-	84.0	2.12	1.75

By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

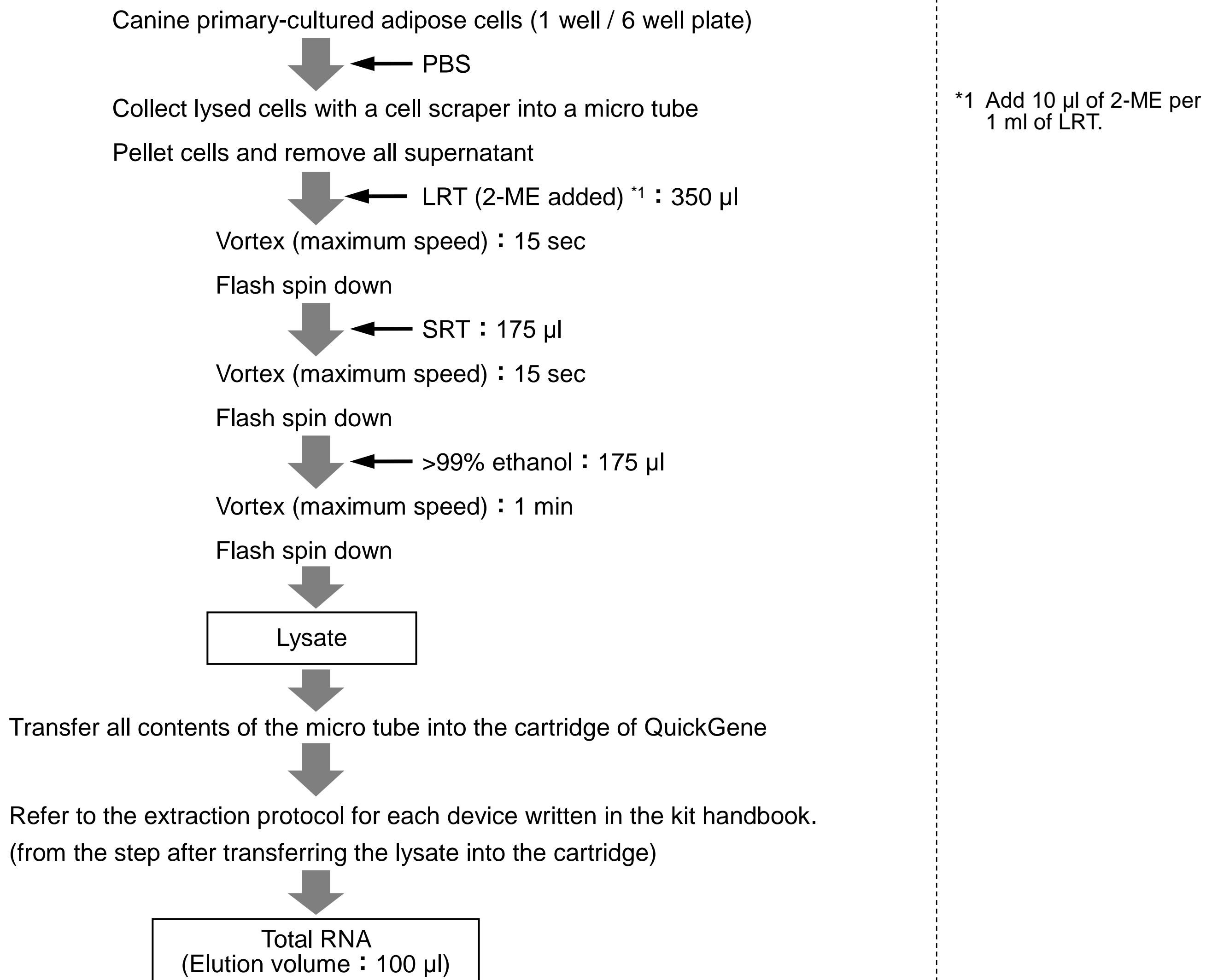
Common protocol is usable for the following

Cultured HeLa Cells, Cultured HEK293 Cells, Cultured COS-7 Cells

RG-16

Total RNA Extraction from Primary-Cultured Adipose Cells of Canine

Protocol



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).

KURABO

Results

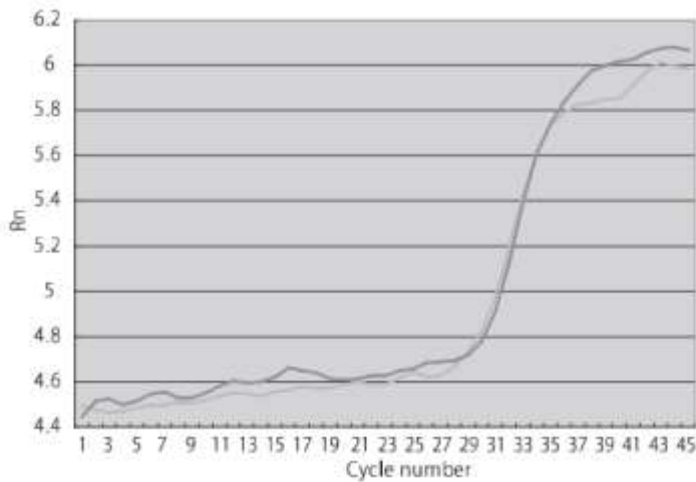
The yield of total RNA / Protein contamination：A260/280

Sample	Number of cells	Protocol	Yield (μg)	A260/280
Adipose Cells of Canine	1 well / 6 well plate	QuickGene	7.9	2.04
		A Company	1.3	2.67

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) on total RNA extracted from canine primary- cultured adipose cells using QuickGene system.



Both are data for total RNA extracted with QuickGene system.

Common protocol is usable for the following

No Data

RG-17

Total RNA Isolation from Cultured HuH-7 Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

↓

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

↓ ← >99% ethanol：100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓ ← >99% ethanol：180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

Other

- PCR

PCR succeeded

Common protocol is usable for the following

Cultured MCF-7 Cells, Cultured Smooth muscle Cells, Cultured PC12 Cells, Cultured Porcine fat Cells, Cultured Lens epithelial Cells, Cultured Periodontal ligament Cells

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).

RG-18

Total RNA Isolation from Cultured MCF-7 Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

The yield of total RNA / Protein contamination : A260/280 / Chaotropic salt contamination : A260/230

Sample	Number of cells	Yield (µg)	A260/280	A260/230
MCF-7 Cells	1.0 x 10^6	9.7	2.06	2.10

Common protocol is usable for the following

Cultured MCF-7 Cells, Cultured Smooth muscle Cells, Cultured PC12 Cells, Cultured Porcine fat Cells, Cultured Lens epithelial Cells, Cultured Periodontal ligament Cells

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).

KURABO

RG-19

Total RNA Isolation from Cultured PC12 Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

↓

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

↓ ← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓ ← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

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

Sample	Number of cells	Yield (µg)	A260/280
PC12 Cells	1.0 x 10^6	About 20.0	1.75

Common protocol is usable for the following

Cultured MCF-7 Cells, HuH-7 Cells, Cultured Smooth muscle Cells, Cultured Porcine fat Cells, Cultured Lens epithelial Cells, Cultured Periodontal ligament Cells

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).

RG-20

Total RNA Isolation from Cultured Smooth muscle Cells (Lysing directly in culture dish)

Protocol

Cell preparation

Number of cells : maximum amount is 1 well / 6 well Plate (3.5 cm dish plate) or 1 x 10^6 cells.
Completely aspirate cell-culture medium, wash cells with PBS, then aspirate PBS completely

↓ ← LRC (2-ME added) *1 : 520 µl

Scrape to lyse the cells for - 30 sec with a cell scraper or the like.

↓

Collect the cell lysate to 1.5 ml micro tube.

Vortex (maximum speed) : 1 min & Flash spin down

↓ ← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓ ← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells, HuH-7 Cells, Cultured PC12 Cells, Cultured Porcine fat Cells, Cultured Lens epithelial Cells, Cultured Periodontal ligament Cells

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).

KURABO

RG-21

Total RNA Extraction from Cultured Cells for DNA chip "Genopal®"

Protocol

Pellet cells and remove all supernatant by aspiration
(Do not use more than 1 x 10^6 cells)

← PBS : 20 µl (For frozen cell pellet)

Loosen the cell pellet by flicking the tube

← LRC (2-ME added) [*1] : 520 µl

Homogenization : Vortex method

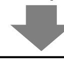
Vortex (maximum speed) : 1 min & Flash spin down

← >99% ethanol : 100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

← >99% ethanol : 180 µl

Vortex (maximum speed) : 15 sec & 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)

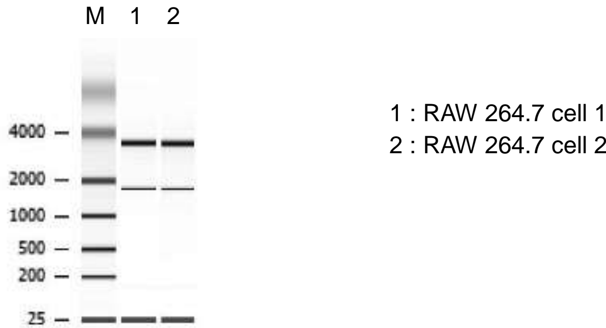
*1 Add 10 µl of 2-ME per 1 ml of LRP.

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).

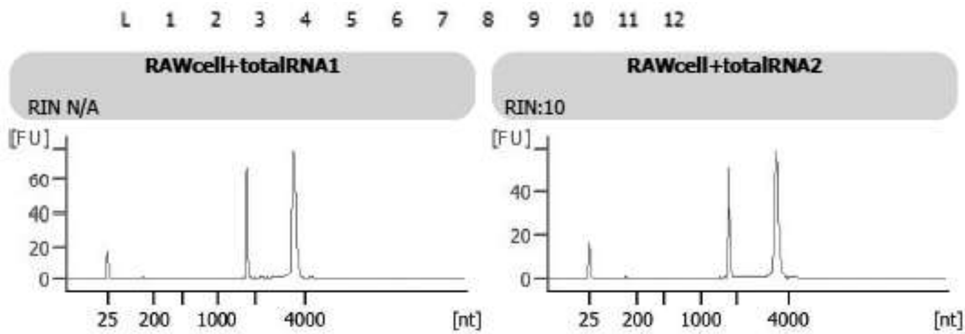
Results

Electrophoregram

Electrophoresis was performed with total RNA extracted from cultured RAW 264.7 cells using QuickGene system.



2100 Bioanalyzer (Agilent Technologies, Inc.)



The yield of total RNA

Sample	No.	Yield (μg)
RAW 264.7	1	38.0
	2	30.0

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).

KURABO

QuickGene

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).

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