Chapter 3-XVII

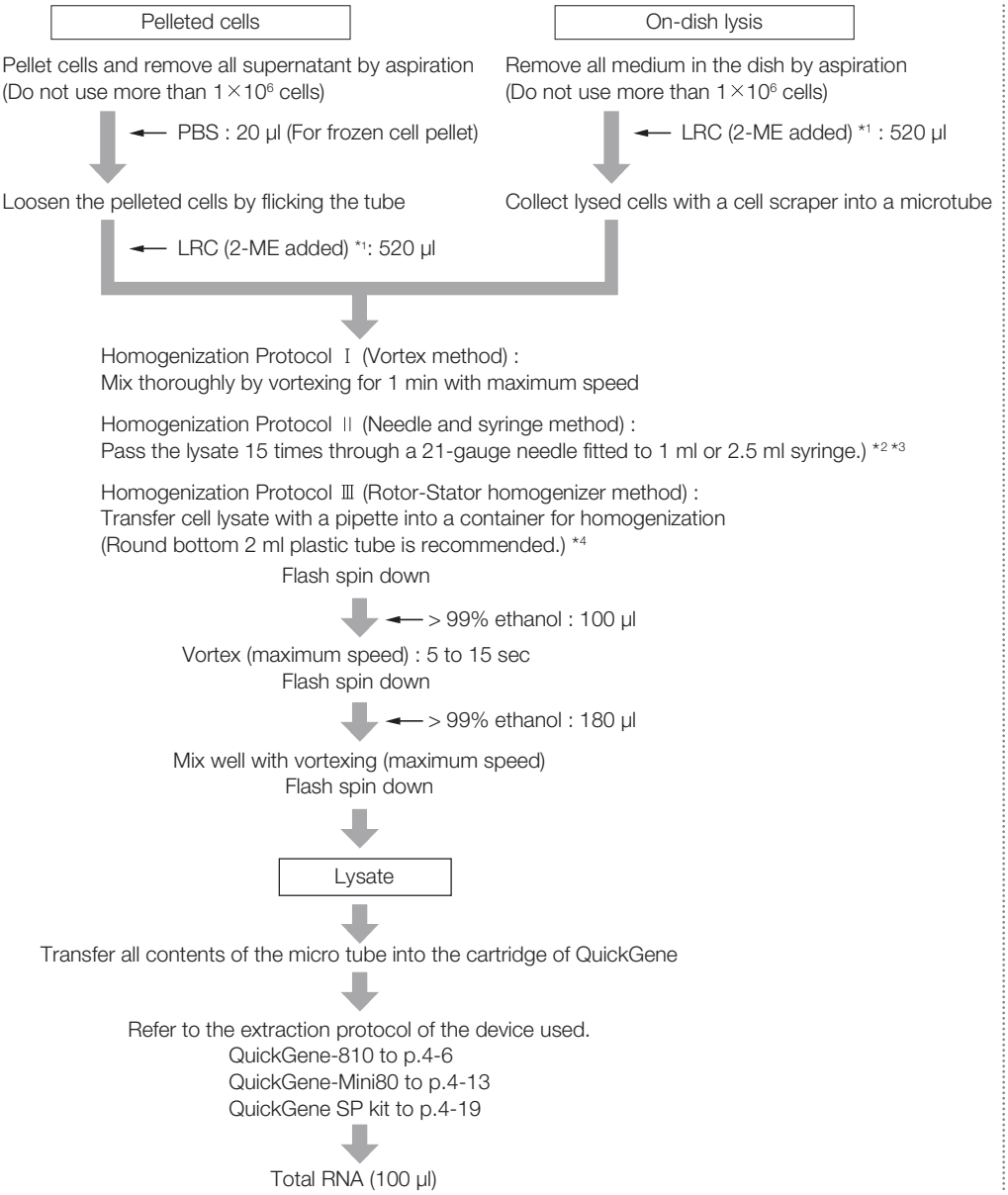
Total RNA Extraction from Cultured Cell

RG-1

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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

Protocol



*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.

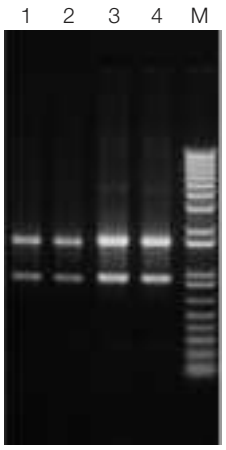
*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

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

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 : 6cm dish, Homogenization protocol Ⅲ
M : Ready Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)

	Number of cells	Homogenization protocol	Yield(μg)
COS-7	0.3×10^6	Ⅱ	13.6
	0.8×10^6	Ⅲ	34.4

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
COS-7	0.3×10^6	Ⅱ	2.19
	0.8×10^6	Ⅲ	1.96

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
COS-7	0.3×10^6	Ⅱ	2.19
	0.8×10^6	Ⅲ	2.17

Other

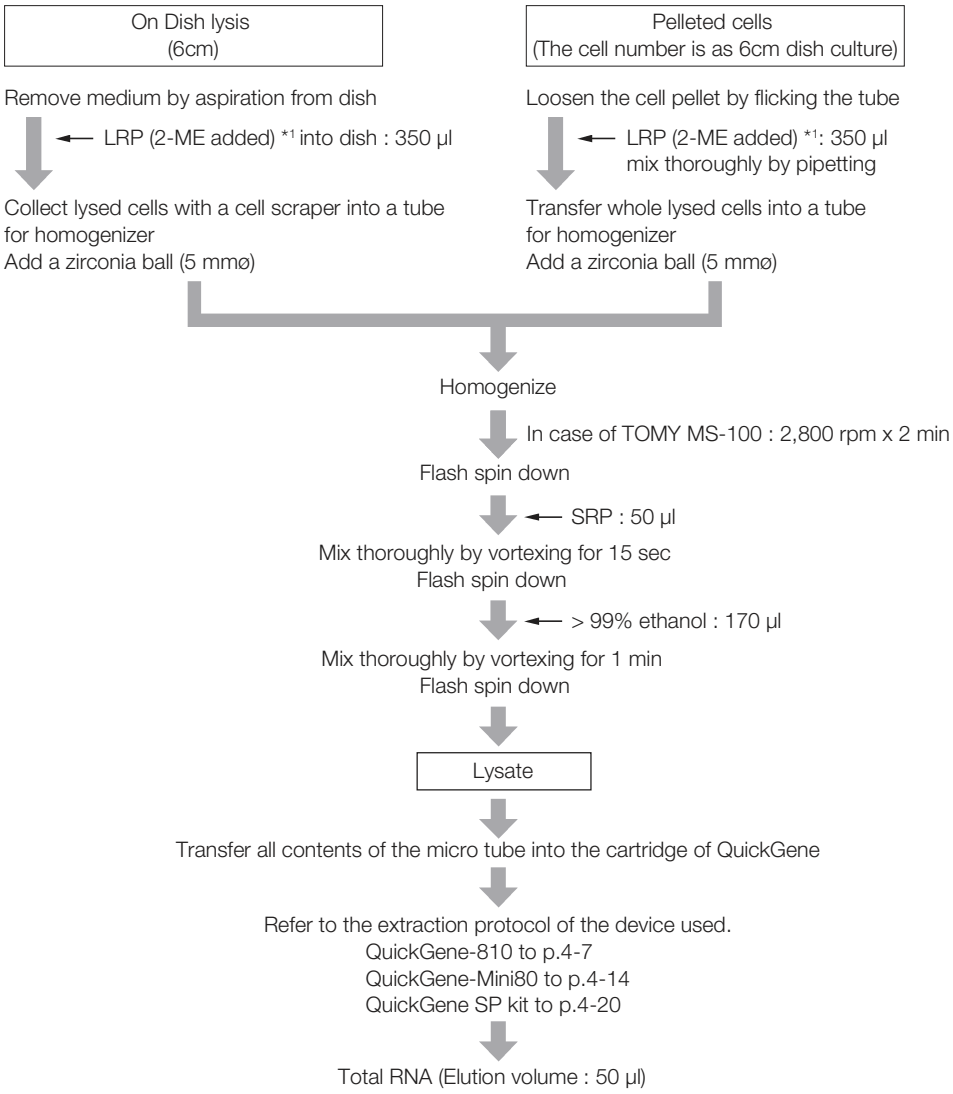
No Data

Common protocol is usable for the following

Cultured HeLa Cells (For $\sim 1 \times 10^6$ cells), Cultured HEK293 Cells (For $\sim 1 \times 10^6$ cells), Cultured NIH/3T3 Cells (For $\sim 1 \times 10^6$ cells)

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 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA (with DNase treatment)

Cell Line	Number of cells (x 10^6cells)	Yield (μg)	
		QuickGene	Spin column method (A company)
COS-7	1.0	42.3	51.4

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

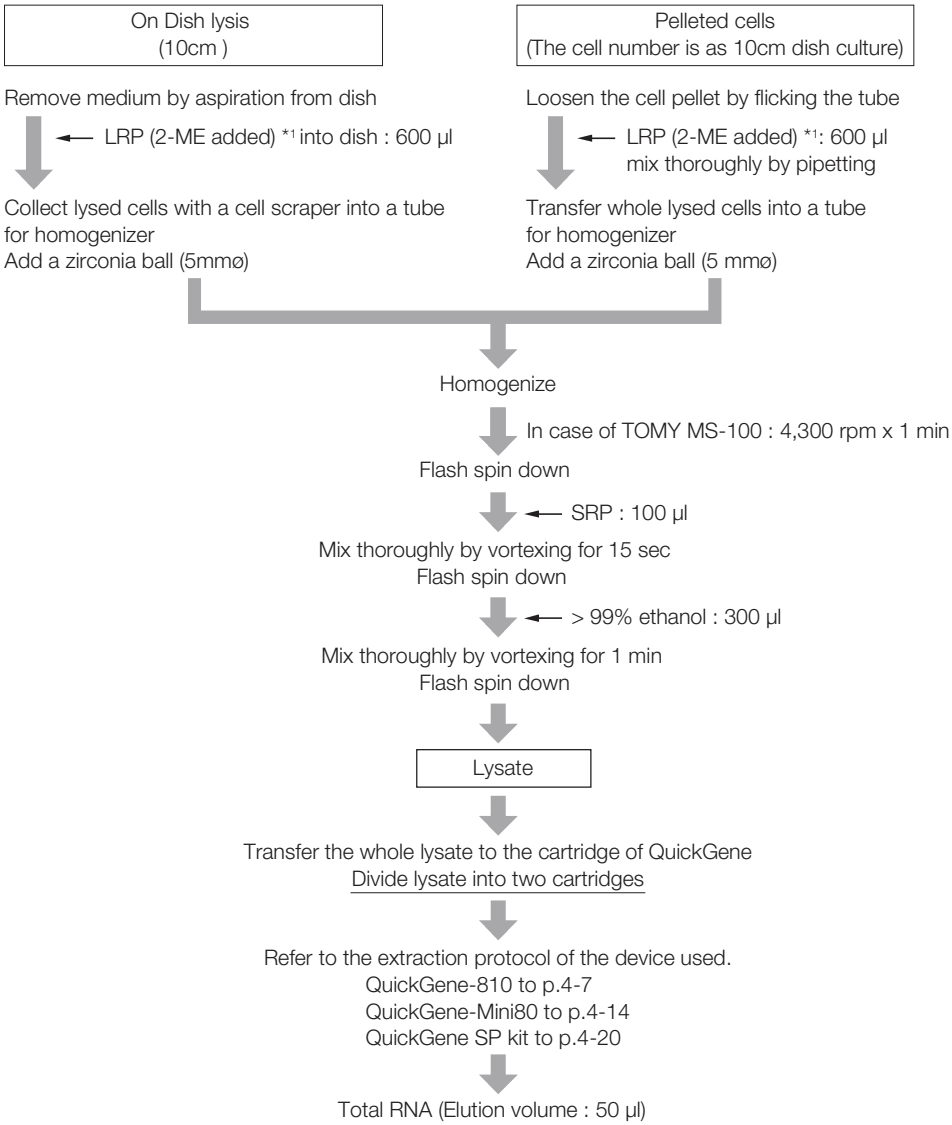
Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

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

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

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (µg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
COS-7	2.5	104.2	98.2	90.0	79.0

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
COS-7	2.5	2.12	1.97	2.12	2.05

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

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
COS-7	2.5	2.11	2.03	1.94	2.19

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

Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

RG-3

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from Cultured ES Cells

Protocol

Pelleted cells

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 homogenizerfor

Homogenize : Mix thoroughly by vortexing for 1 min with maximum speed

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 the whole lysate to the cartridge of QuickGene divide lysate into two cartridges

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-7
QuickGene-Mini80 to p.4-14
QuickGene SP kit to p.4-20

Total RNA (Elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

Electropherogram

No Data

The yield of total RNA

Number of ES cells	Yield(µg)
2×10^6 cells	41.4 (2 cartridges)

Protein contamination : A260/280

Number of ES cells	A260/280
2×10^6 cells	2.1

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

Total RNA Extraction from Cultured HEK293 Cells (For ~ 1 × 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 microtube

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) : 5 to 15 sec
Flash spin down

← > 99% ethanol : 180 µl

Mix well with vortexing (maximum speed)
Flash spin down

Lysate

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

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

Total RNA (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.

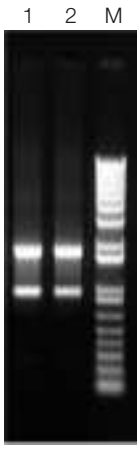
*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

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

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)

	Number of cells	Homogenization protocol	Yield(μg)
HEK293	2.1×10^6	Ⅱ	30.4

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
HEK293	2.1×10^6	Ⅱ	2.27

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
HEK293	2.1×10^6	Ⅱ	2.14

Other

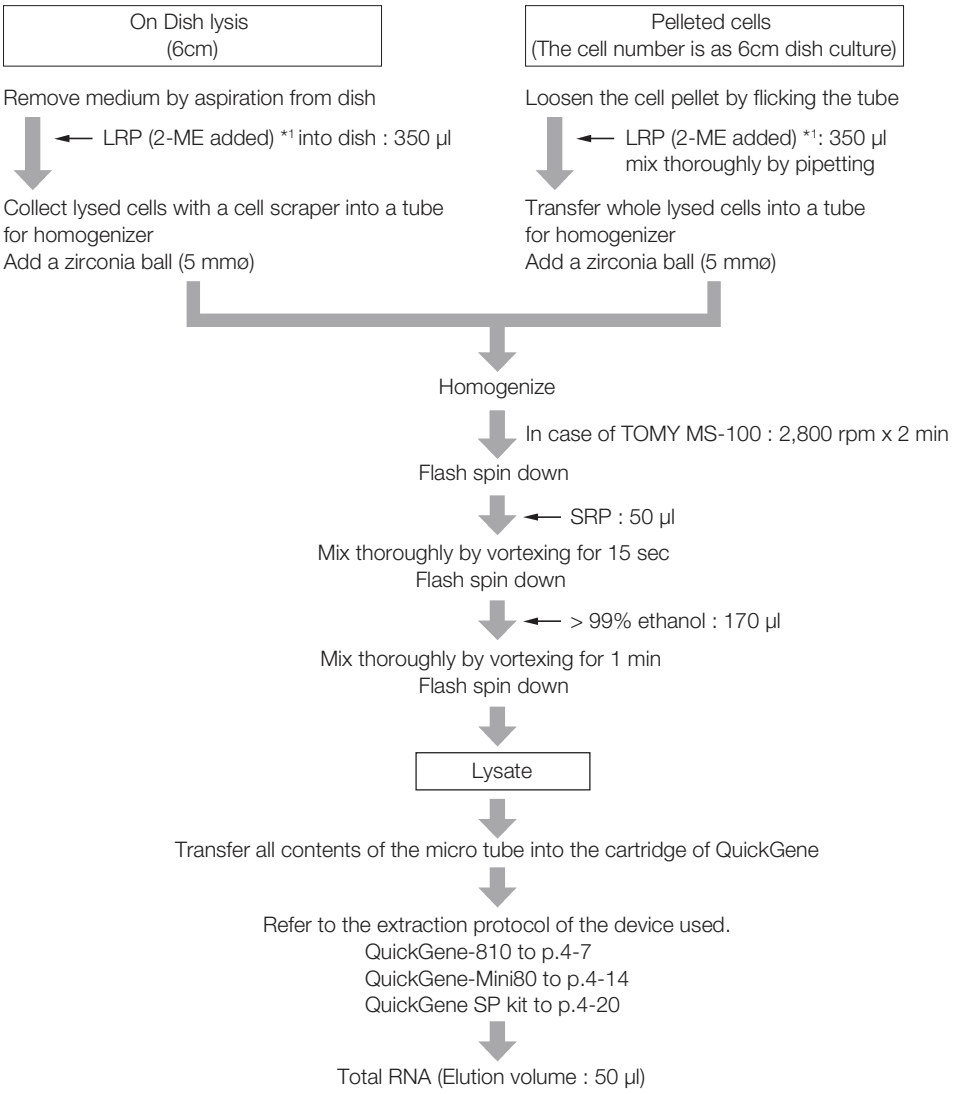
No Data

Common protocol is usable for the following

Cultured COS-7 Cells (For ～1×10^6 cells), Cultured HeLa Cells (For ～1×10^6 cells), Cultured NIH/3T3 Cells (For ～1×10^6 cells)

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.

Results

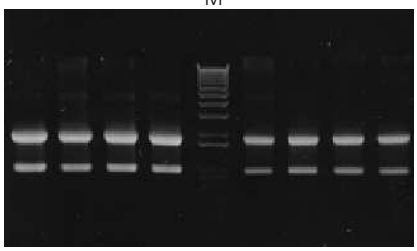
Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

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

HEK293 (5 x 10^6cells)

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



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

The yield of total RNA (with DNase treatment)

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

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

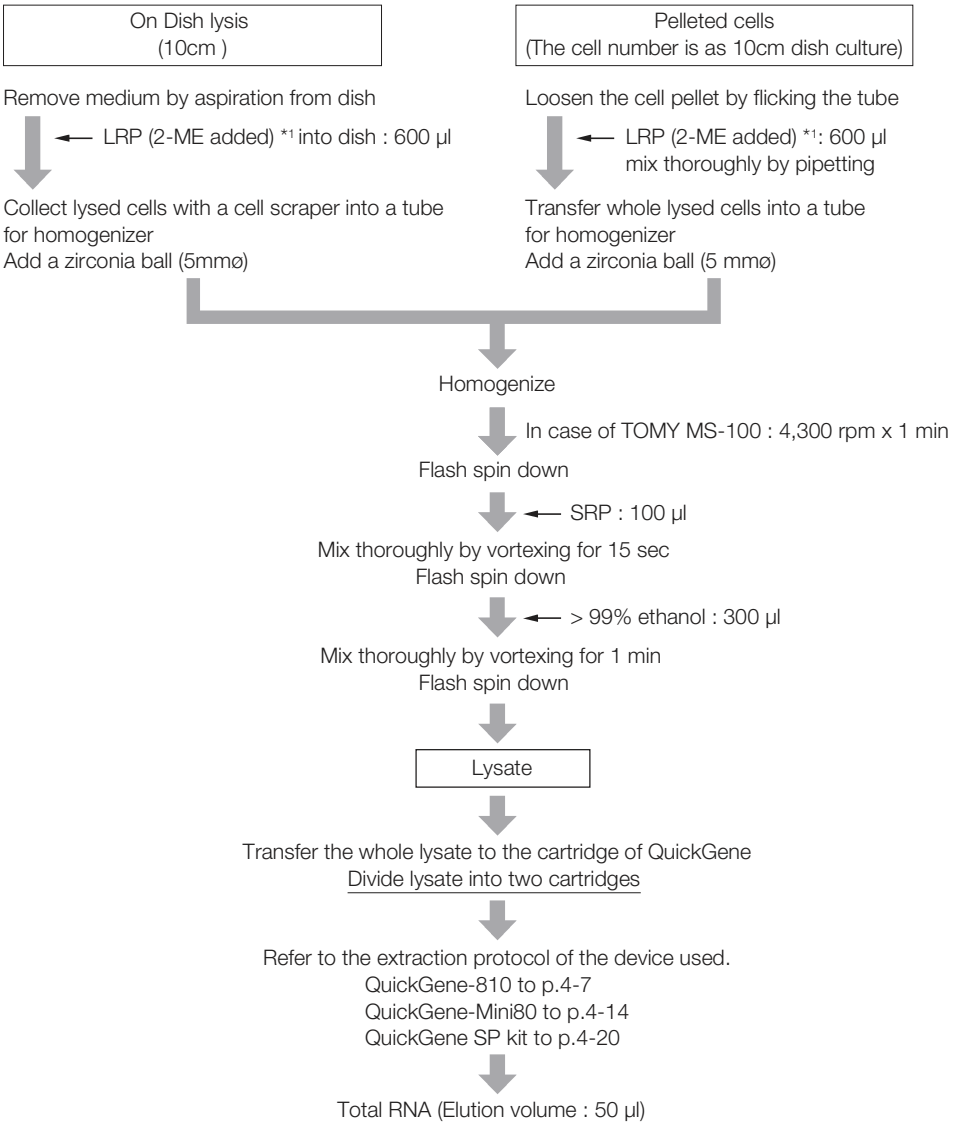
Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

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

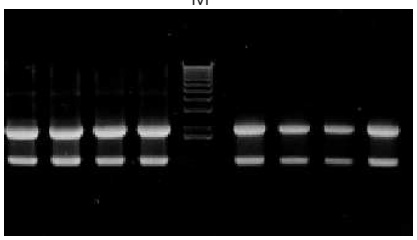
Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

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

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	5.0-8.0	175.3	92.2	160.3	101.0

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	5.0-8.0	2.29	2.11	2.27	2.11

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

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	5.0-8.0	2.12	2.16	2.11	2.18

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

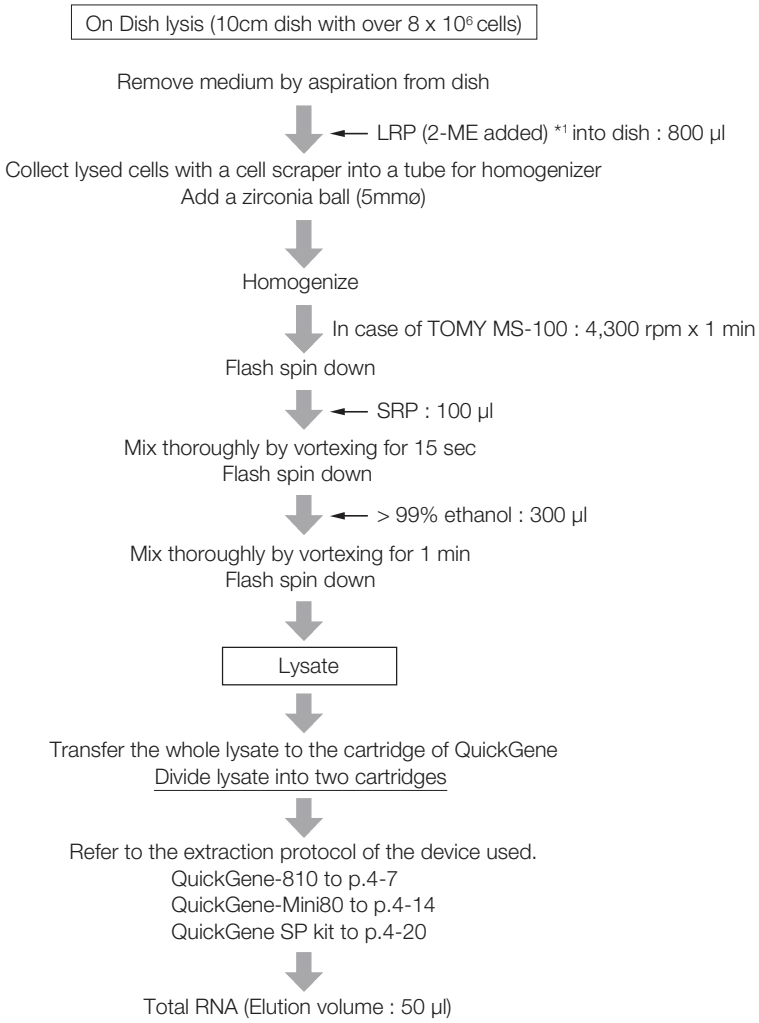
Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

Protocol B'



*1 2-Merc aptoethanol (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).

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (µg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	149.5	133.1	94.9	102.3

By use of QuickGene system total RNA amount necessary for microarray, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	1.95	2.04	1.98	2.02

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

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	2.14	2.14	1.88	2.17

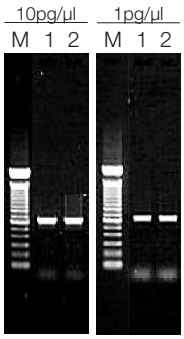
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

• RT-PCR (with DNase treatment)

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

HEK293 (12 x 10^6cells)



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

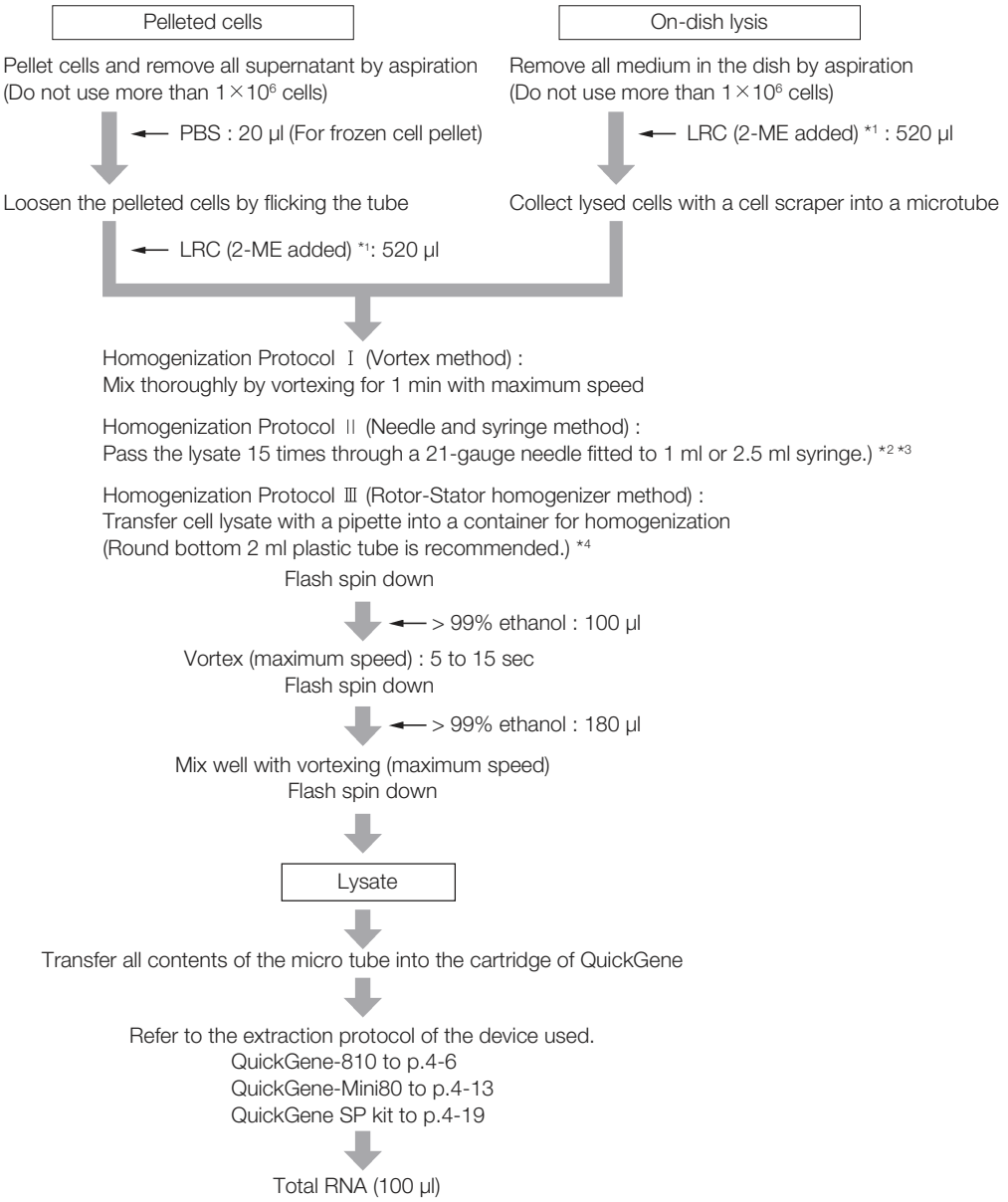
For RT-PCR performed on total RNA (1pg/µl), similar electrophoretic bands of the amplification products were detected for both kits.

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

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

Protocol



*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.

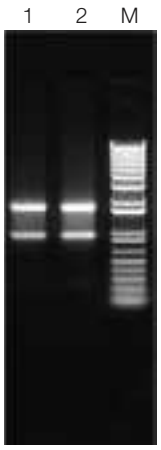
*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

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

Results

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)

	Number of cells	Homogenization protocol	Yield(μg)
HeLa	1.2×10^6	Ⅱ	28.1

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
HeLa	1.2×10^6	Ⅱ	2.28

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
HeLa	1.2×10^6	Ⅱ	2.21

Other

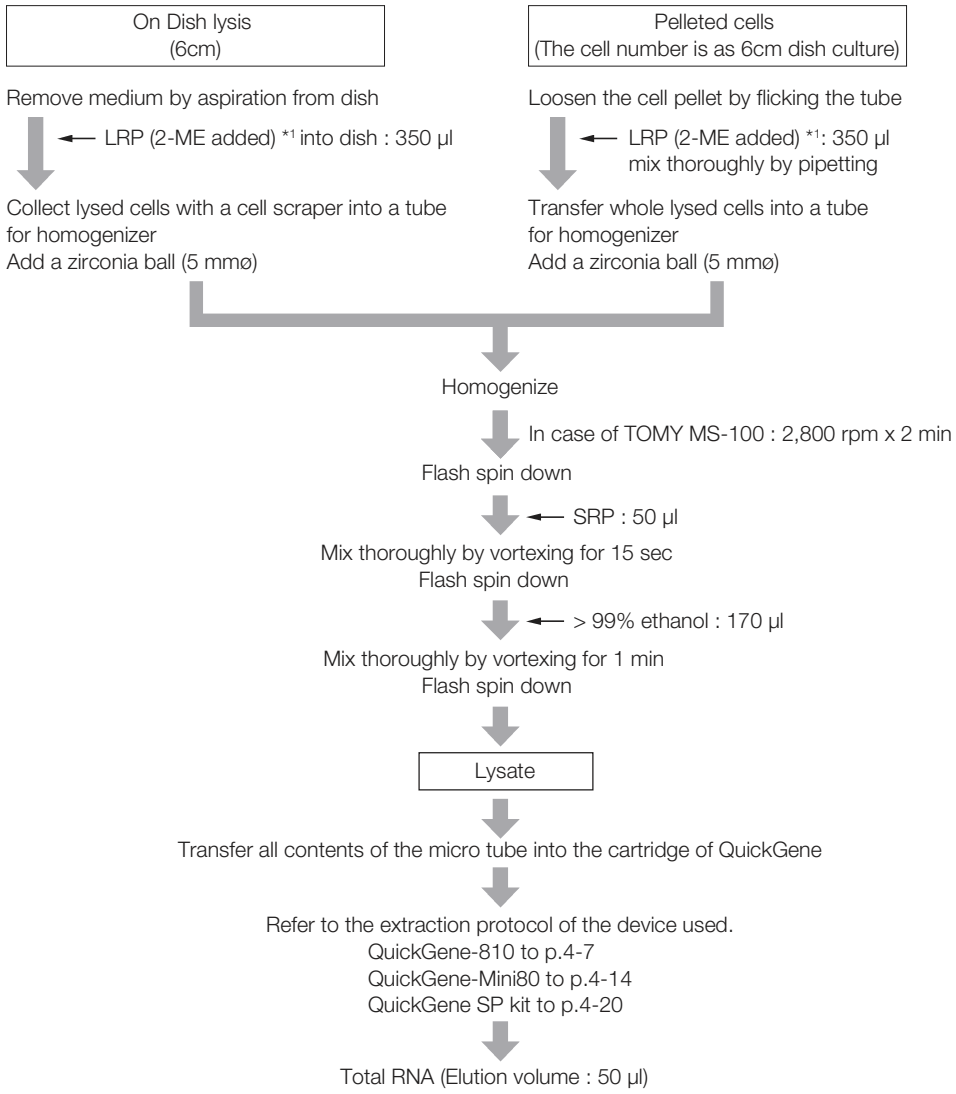
No Data

Common protocol is usable for the following

Cultured COS-7 Cells (For ～1×10^6 cells), Cultured HEK293 Cells (For ～1×10^6 cells), Cultured NIH/3T3 Cells (For ～1×10^6 cells)

Total RNA Extraction from Cultured HeLa 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 2-ME per 1ml of LRP.

Results

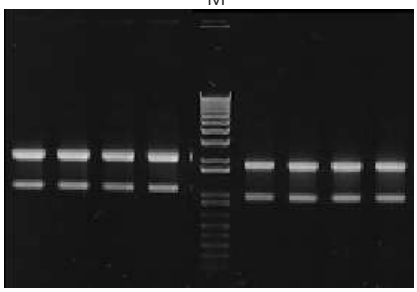
Lysing adherent cells directly in 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

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

HeLa (2 x 10^6cells)

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



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

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (µg)	
		QuickGene	Spin column method (A company)
HeLa	2.0	47.2	46.1

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

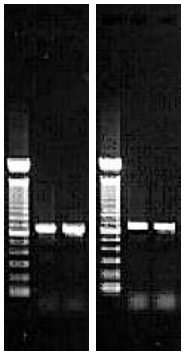
Other

• RT-PCR (with DNase treatment)

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

HeLa (6cm dish)

10pg/µl M 1 2 1pg/µl M 1 2



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

Common protocol is usable for the following

Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

Protocol B

On Dish lysis (10cm)	Pelleted cells (The cell number is as 10cm dish culture)
Remove medium by aspiration from dish	Loosen the cell pellet by flicking the tube
← LRP (2-ME added) *1 into dish : 600 µl	← LRP (2-ME added) *1: 600 µl mix thoroughly by pipetting
Collect lysed cells with a cell scraper into a tube for homogenizer Add a zirconia ball (5mmø)	Transfer whole lysed cells into a tube for homogenizer Add a zirconia ball (5 mmø)

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

↓ Homogenize

↓ In case of 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 the whole lysate to the cartridge of QuickGene
Divide lysate into two cartridges

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-7
QuickGene-Mini80 to p.4-14
QuickGene SP kit to p.4-20

↓

Total RNA (Elution volume : 50 µl)

Results

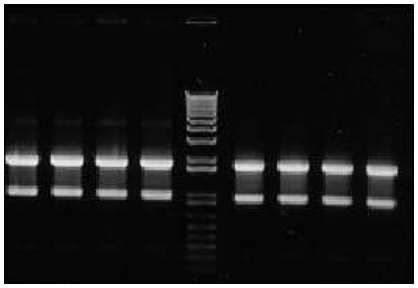
Lysing adherent cells directly in 10cm dish, or lysing pelleted floating cells of 10cm dish, total RNA was isolated.

Electropherogram

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

HeLa (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

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	129.0	115.7	122.0	104.0

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	2.20	1.99	2.20	2.02

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

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	2.18	2.10	2.05	2.12

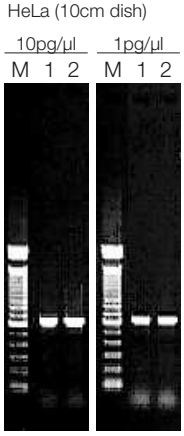
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

• RT-PCR

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

HeLa (10cm dish)



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

Common protocol is usable for the following

Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish), NIH/3T3 Cells (For cells cultured in 6cm or 10cm dish)

Total RNA Extraction from Cultured HL60 Cells (For ～1 × 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

Vortex (maximum speed) : 1 min
Flash spin down

Vortex (maximum speed) : 5 to 15 sec
Flash spin down

Mix well with vortexing (maximum speed)
Flash spin down

Lysate

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

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

Total RNA (100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

	Number of cells	Yield(µg)
HL60	1.0 × 10^6	9.7

Protein contamination : A260/280

	Number of cells	A260/280
HL60	1.0 × 10^6	1.88

Chaotropic salt contamination : A260/230

	Number of cells	A260/230
HL60	1.0 × 10^6	2.08

Other

No Data

Common protocol is usable for the following

No Data

RG-9

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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 × 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 tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

[Lysate]

↓

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

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

Number of lens epithelial cells	A260/280
1 × 10^6 cells	1.77

Chaotropic salt contamination : A260/230

No Data

Other

No Data

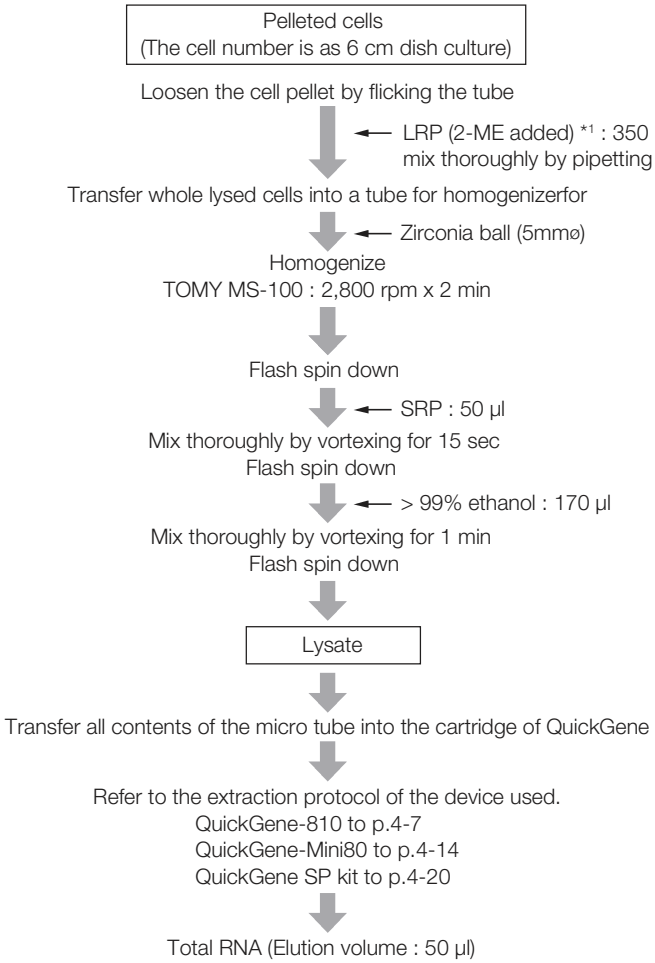
Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from Cultured Lymphocytes

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRP.

Results

Electropherogram

No Data

The yield of total RNA

Number of lymphocytes	Yield(µg)
1×10^6 cells	13.4

Protein contamination : A260/280

Number of lymphocytes	A260/280
1×10^6 cells	1.67

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

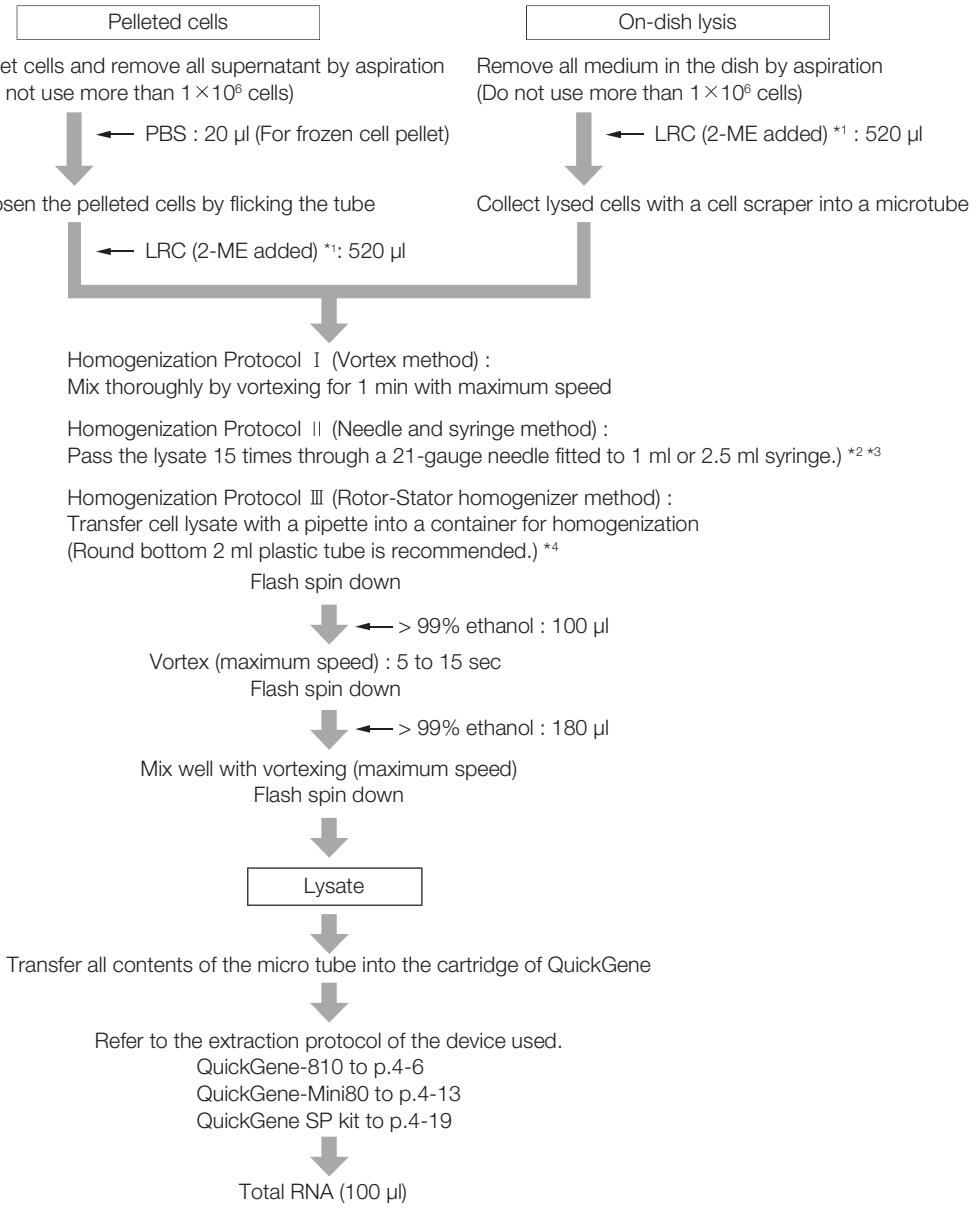
No Data

RG-11

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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

Protocol



*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.

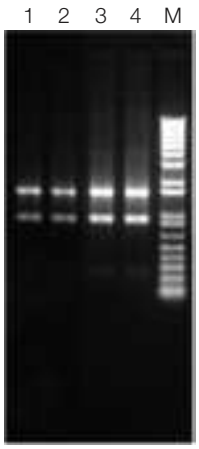
*3 When you use the sample which may be infected, be careful of the handling of a needle enough.

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

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 : 6cm dish, Homogenization protocol Ⅱ
M : Ready-Load 1kb Plus DNA Ladder : Invitrogen

The yield of total RNA (with DNase treatment)

	Number of cells	Homogenization protocol	Yield(μg)
NIH/3T3	0.3×10^6	Ⅰ	15.6
	1.2×10^6	Ⅱ	22.6

Protein contamination : A260/280

	Number of cells	Homogenization protocol	Purity
			Protein contamination A260/280
NIH/3T3	0.3×10^6	Ⅰ	2.17
	1.2×10^6	Ⅱ	2.26

Chaotropic salt contamination : A260/230

	Number of cells	Homogenization protocol	Purity
			Chaotropic salt contamination A260/230
NIH/3T3	0.3×10^6	Ⅰ	2.18
	1.2×10^6	Ⅱ	2.22

Other

No Data

Common protocol is usable for the following

Cultured COS-7 Cells (For ～1×10^6 cells), Cultured HeLa Cells (For ～1×10^6 cells), Cultured HEK293 Cells (For ～1×10^6 cells)

RG-12

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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 × 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 tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

[Lysate]

↓

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

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Number of periodontal ligament cells	Yield(µg)
about 1 × 10^5 cells	1.2

Protein contamination : A260/280

Number of periodontal ligament cells	A260/280
about 1 × 10^5 cells	1.9

Chaotropic salt contamination : A260/230

Number of periodontal ligament cells	A260/230
about 1 × 10^5 cells	1.2

Other

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish)

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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 × 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 tube.)
Mix thoroughly by vortexing for 1 min with maximum speed
↓ ← >99% ethanol : 100 µl
Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down
↓ ← >99% ethanol : 180 µl
Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19
↓
Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Kind of cells	Yield(µg)
differentiated cells	0.6
undifferentiated cells	1.2

Protein contamination : A260/280

Kind of cells	A260/280
differentiated cells	2.09
undifferentiated cells	2.07

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

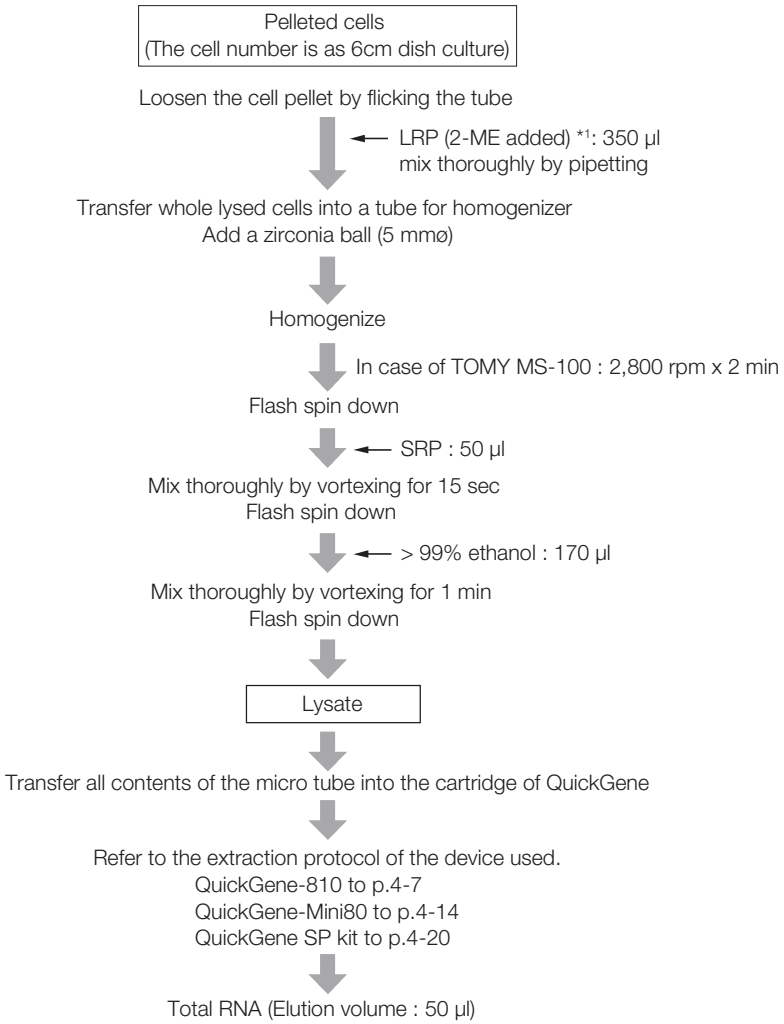
Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

RG-14

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from HL60 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 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA (with DNase treatment)

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

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

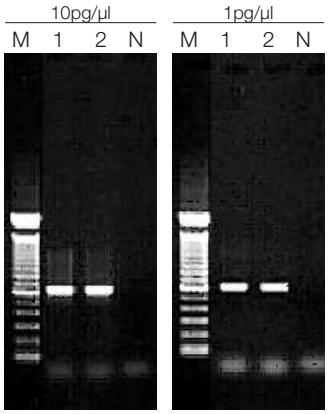
No Data

Other

• RT-PCR (with DNase treatment)

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

HL60 (5 x 10^6cells)



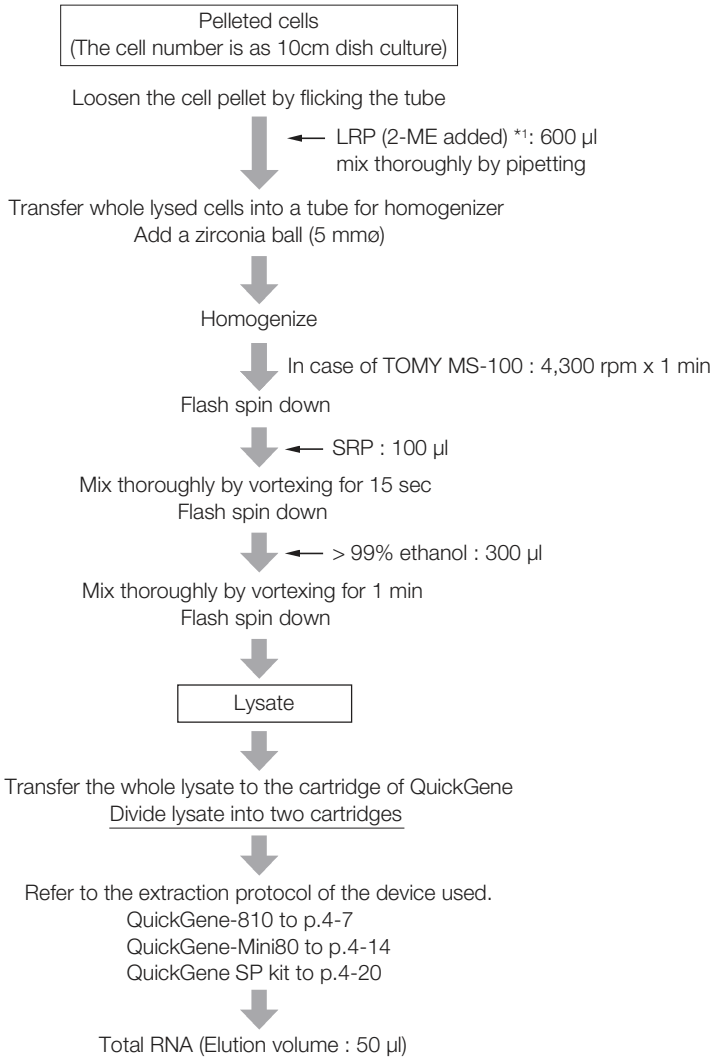
M : Marker (100bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A company)
N : Negative control

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

Common protocol is usable for the following

No Data

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

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

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (µg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HL60	15.0	167.3	154.4	144.4	140.5

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HL60	15.0	1.92	1.85	2.18	2.09

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

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HL60	15.0	2.17	2.15	2.18	2.12

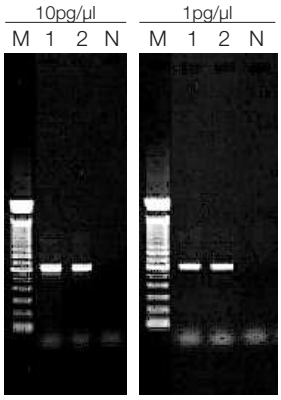
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

Other

• RT-PCR

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

HL60 (15 x 10^6cells)



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

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

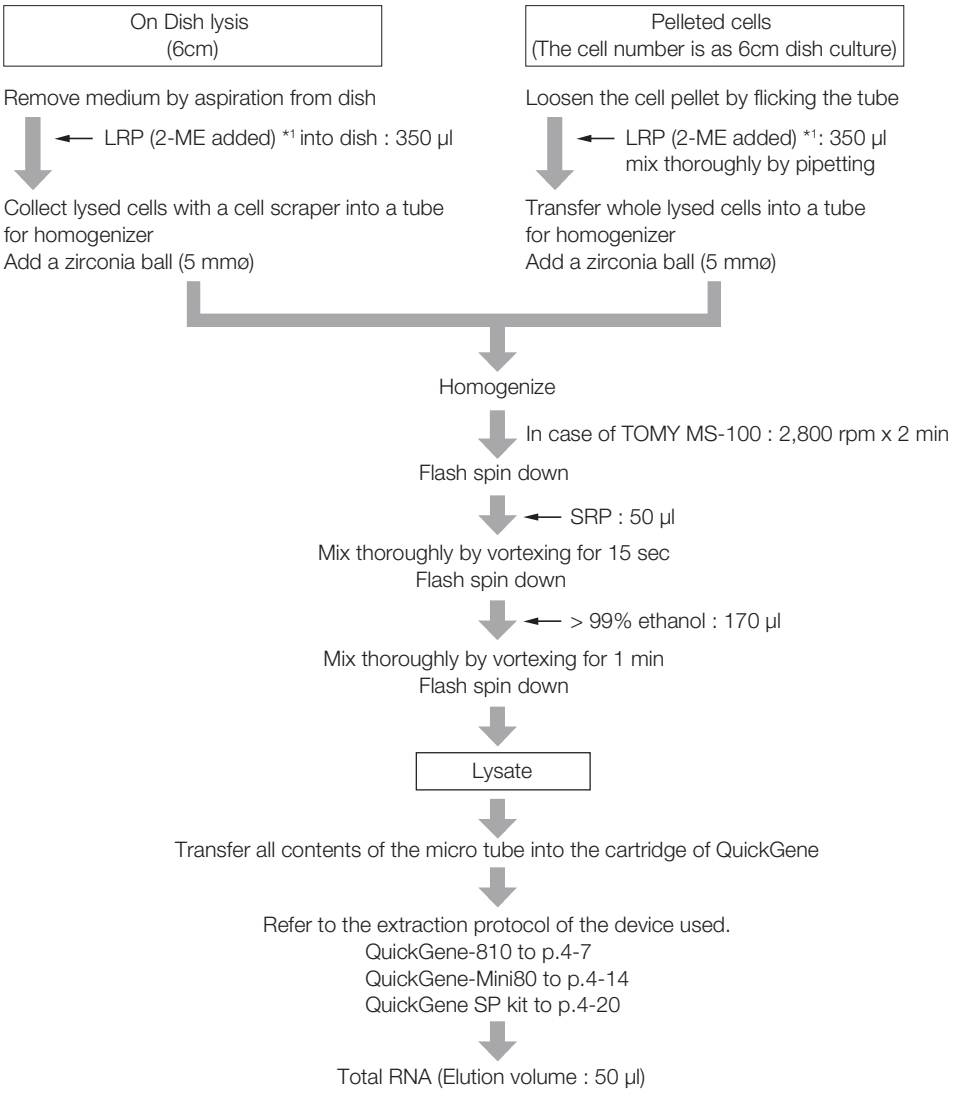
Common protocol is usable for the following

No Data

QuickGene RNA cultured cell HC kit S (RC-S2)
QuickGene SP kit RNA cultured cell HC (SP-RC2)

Total RNA Extraction from NIH/3T3 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 6cm dish, or lysing pelleted floating cells of 6cm dish, total RNA was isolated.

Electropherogram

No Data

The yield of total RNA (with DNase treatment)

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

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

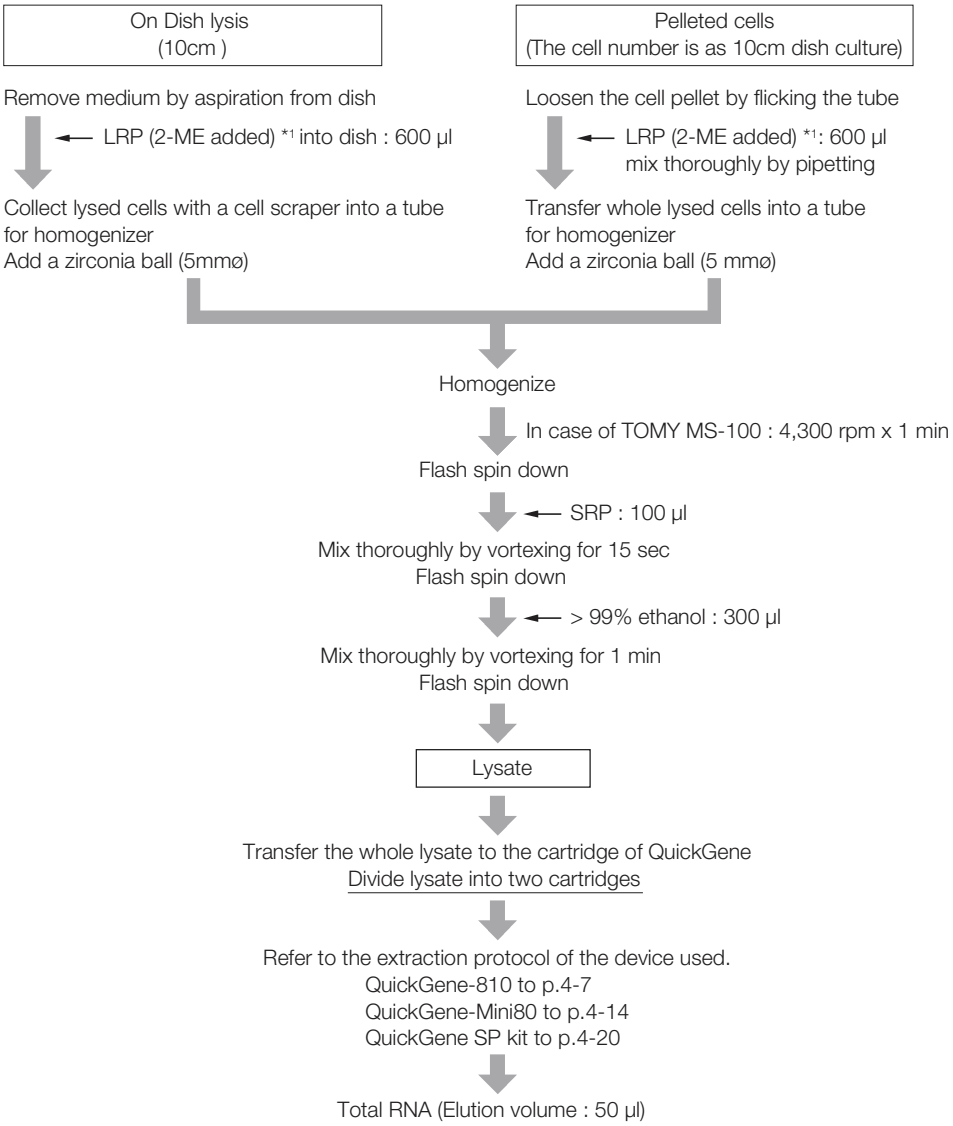
Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)

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

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

Electropherogram

No Data

The yield of total RNA

Cell Line	Number of cells (x 10^6cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
NIH / 3T3	4.5	89.4	100.2	79.0	84.0

By use of QuickGene system total RNA amount necessary for microarry, Northern blotting and so on can be obtained.

Protein contamination : A260/280

Cell Line	Number of cells (x 10^6cells)	A260/280			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
NIH / 3T3	4.5	2.19	2.02	2.17	2.12

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

Chaotropic salt contamination : A260/230

Cell Line	Number of cells (x 10^6cells)	A260/230			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
NIH / 3T3	4.5	2.02	2.26	1.94	1.75

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

Other

No Data

Common protocol is usable for the following

Cultured HeLa Cells (For cells cultured in 6cm or 10cm dish), Cultured HEK293 Cells (For cells cultured in 6cm or 10cm dish), Cultured COS-7 Cells (For cells cultured in 6cm or 10cm dish)

Total RNA Extraction from Primary-Cultured Adipose Cells of Canine

Protocol

Canine primary-cultured adipose cells (1well/6well plate)

↓ ← PBS

Collect lysed cells with a cell scraper into a microtube
Pellet cells and remove all supernatant

↓ ← LRT (2-ME added) *1 : 350 µl

Vortex (maximum speed) : 1 min
Flash spin down

↓ ← SRT : 175 µl

Vortex (maximum speed) : 15 sec
Flash spin down

↓ ← >99% ethanol : 175 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 of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

Total RNA (Elution volume : 100 µl)

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

Results

Electropherogram

No Data

The yield of total RNA

Number of cells	QuickGene	Competitor A kit
1 well / 6 well plate	7.9 µg	1.3 µg

Protein contamination : A260/280

Number of cells	QuickGene	Competitor A kit
1 well / 6 well plate	2.04	2.67

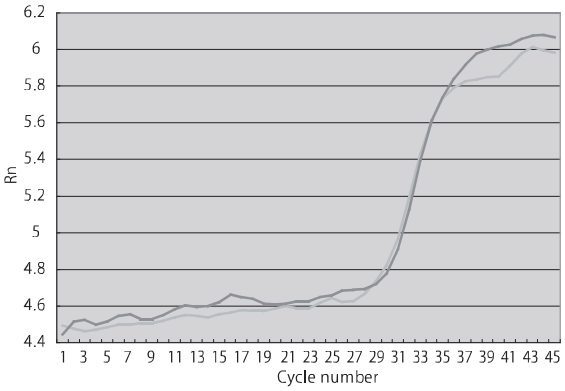
Chaotropic salt contamination : A260/230

No Data

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

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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 × 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 tube.)
Mix thoroughly by vortexing for 1 min with maximum speed
↓ ← >99% ethanol : 100 µl
Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down
↓ ← >99% ethanol : 180 µl
Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19
↓
Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

• PCR

PCR succeeded

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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 × 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 tube.)
Mix thoroughly by vortexing for 1 min with maximum speed
↓ ← >99% ethanol : 100 µl
Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down
↓ ← >99% ethanol : 180 µl
Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19
↓
Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Number of MCF-7 cells	Yield(µg)
1 × 10^6 cells	9.7

Protein contamination : A260/280

Number of MCF-7 cells	A260/280
1 × 10^6 cells	2.06

Chaotropic salt contamination : A260/230

Number of MCF-7 cells	A260/230
1 × 10^6 cells	2.10

Other

No Data

Common protocol is usable for the following

HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

RG-19

QuickGene RNA cultured cell kit S (RC-S)
QuickGene SP kit RNA cultured cell (SP-RC)

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 × 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 tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

↓ ← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

↓

[Lysate]

↓

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

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

↓

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

Number of PC12 cells	Yield(µg)
1 × 10^6 cells	about 20.0

Protein contamination : A260/280

Number of PC12 cells	A260/280
1 × 10^6 cells	1.75

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured Smooth muscle Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

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 × 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 tube.)

Mix thoroughly by vortexing for 1 min with maximum speed

← >99% ethanol : 100 µl

Mix thoroughly by vortexing for 5 - 15 sec with maximum speed
Flash spin down

← >99% ethanol : 180 µl

Mix thoroughly by vortexing (maximum speed) or pipetting
Flash spin down

Lysate

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

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-6
QuickGene-Mini80 to p.4-13
QuickGene SP kit to p.4-19

Total RNA (Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

Results

Electropherogram

No Data

The yield of total RNA

No Data

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

Cultured MCF-7 Cells (Lysing directly in culture dish), HuH-7 Cells (Lysing directly in culture dish), Cultured PC12 Cells (Lysing directly in culture dish), Cultured Porcine fat Cells (Lysing directly in culture dish), Cultured Lens epithelial Cells (Lysing directly in culture dish), Cultured Periodontal ligament Cells (Lysing directly in culture dish)

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 × 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

Homogenization : Vortex method
Mix thoroughly by vortexing for 1 min with maximum speed
Flash spin down

↓ ← >99% ethanol : 100 µl

Vortexing (maximum speed) : 5-15 sec
Flash spin down

↓ ← >99% ethanol : 180 µl

Mix well with vortexing (maximum speed)
Flash spin down

↓

[Lysate]

↓

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

↓

Refer to the extraction protocol of the device used.
QuickGene-810 to p.4-8
QuickGene-Mini80 to p.4-15
QuickGene SP kit to p.4-22

↓

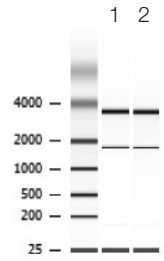
Total RNA (Initial elution volume : 50 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRT.

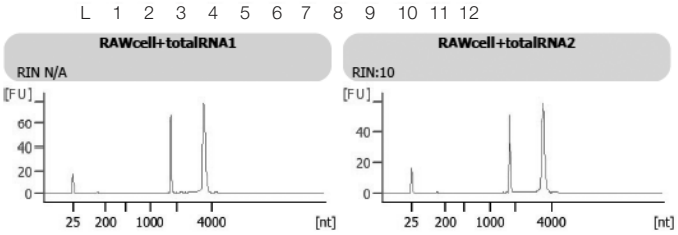
Results

Electropherogram

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



1 : RAW 264.7 cell 1
2 : RAW 264.7 cell 2



2100 Bioanalyzer (Agilent Technologies, Inc.)

The yield of total RNA

sample	Yield(μg)	
	1	2
RAW 264.7	38.0	30.0

Protein contamination : A260/280

No Data

Chaotropic salt contamination : A260/230

No Data

Other

No Data

Common protocol is usable for the following

No Data

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