



QuickGene Series Application Guide

Total RNA Isolation from Cultured Cells

Total RNA Isolation from Cells cultured in 6cm, 10cm Dish

Kit : QuickGene RNA cultured cell HC kit S

Model : QuickGene-810

Summary

- This is the protocol for total RNA isolation from cells cultured in 6cm or 10cm dish. By use of ball mill homogenizer total RNA amount necessary for microarray, Northern blotting and so on can be obtained.

(Treatable number of cells, refer to Table 1.)

Table 1 Dish size, number of cells and yield of total RNA isolated with this kit (homogenized with TOMY MS-100 ; with DNase treatment)

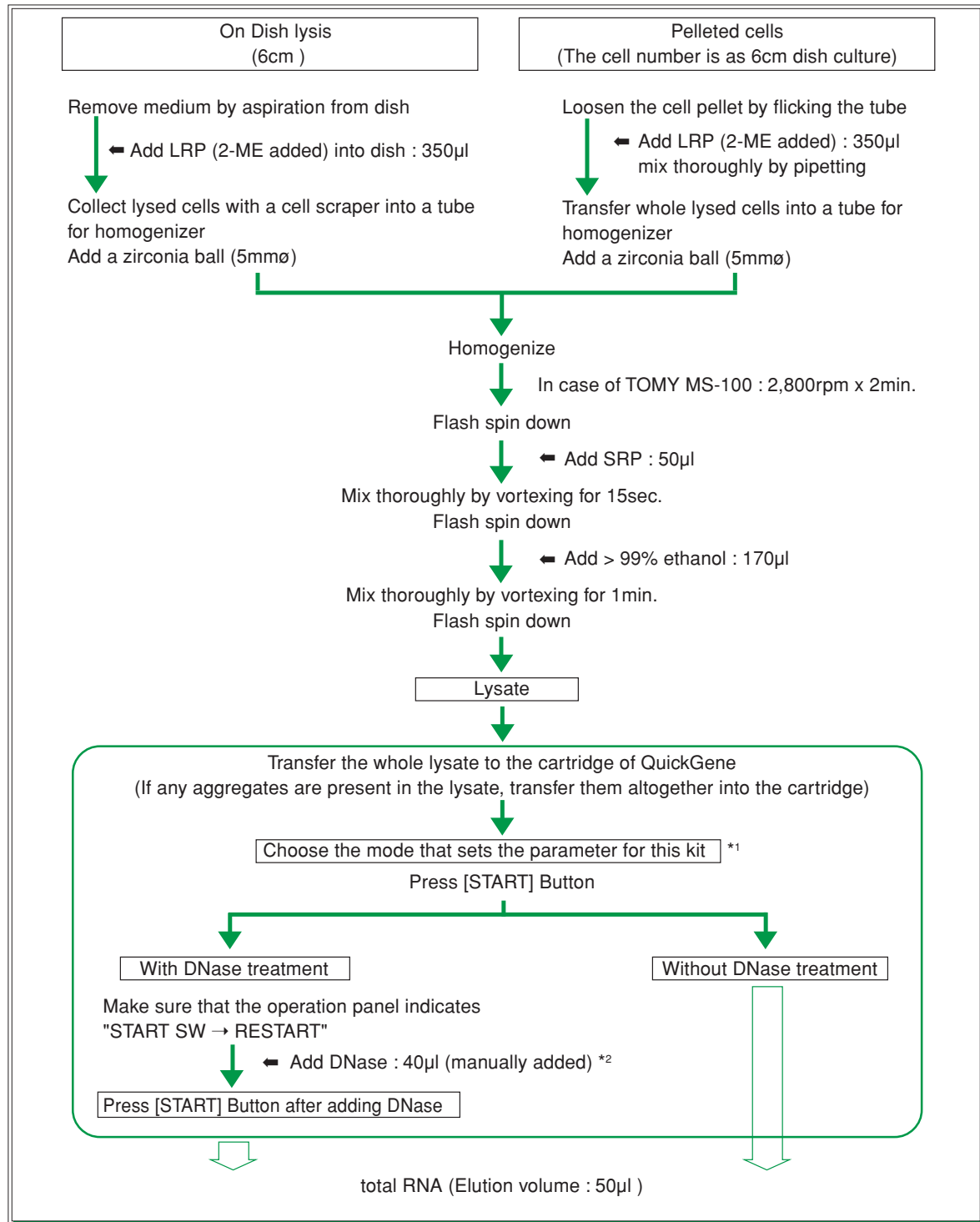
Dish size	Isolation method	Adherent cells								Cells grown in suspension	
		HeLa		HEK293		COS-7		NIH/3T3		HL60	
		Number of cells (x 10 ⁶ cells)	Yield (μg)	Number of cells (x 10 ⁶ cells)	Yield (μg)	Number of cells (x 10 ⁶ cells)	Yield (μg)	Number of cells (x 10 ⁶ cells)	Yield (μg)	Number of cells (x 10 ⁶ cells)	Yield (μg)
6cm	Protocol A	1.5 - 3.5	45 - 70	3.0 - 5.0	40 - 90	0.5 - 1.5	20 - 40	1.0 - 2.5	25 - 30	3.0 - 5.0	25 - 40
10cm	Protocol B	3.5 - 5.5	100 - 150 [80 - 120]	5.0 - 8.0	90 - 150	2.0 - 3.0	100 - 150 [60 - 90]	3.0 - 5.0	80 - 90	5.0 - 15	80 - 150
	Protocol B'	-	-	8.0 - 15	50 - 150	-	-	-	-	-	-

Note 1 : This number means the number of cells cultured by standard method. If excessive number of cells are used, clogging the cartridge may occur.

Note 2 : For isolation from cell type not included in Table 1, start isolation with the number of cells corresponding to subconfluent in 6cm dish, and examine optimal number of cells.

[] : yield for pelleted cells.

Protocol A



*1 : Regarding parameter setting method, please refer to Kit Handbook appendix 1(p.24).

*2 : Please use recommended DNase products. As to the recommended products and solution preparation method, refer to Kit Hand Book.

Example of total RNA isolation from various cultured cells

Protocol A

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

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

● The yield of total RNA (with DNase treatment)

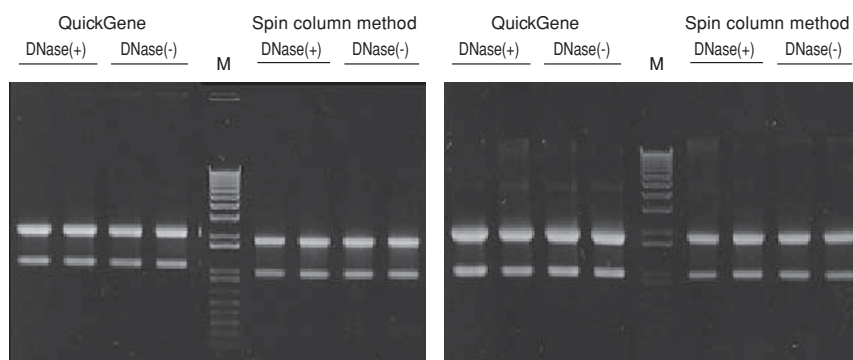
Cell Line	Number of cells (x 10 ⁶ cells)	Yield (μg)	
		QuickGene	Spin colum method(A company)
HeLa	2.0	47.2	46.1
HEK293	5.0	79.1	57.5
COS-7	1.0	42.3	51.4
NIH / 3T3	1.5	27.9	35.7
HL60	5.0	33.1	46.2

● Electrophoresis of total RNA (with DNase treatment)

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

HeLa (2 x 10⁶cells)

HEK293 (5 x 10⁶cells)



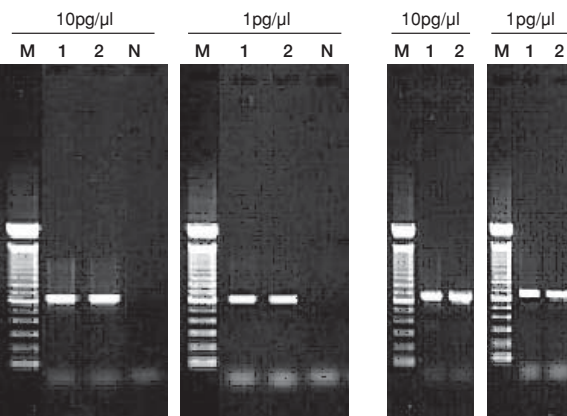
M : Marker (1Kb Plus DNA Ladder : Invitrogen)

● RT-PCR (with DNase treatment)

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

HL60 (5 x 10⁶cells)

HeLa (6cm dish)



M : Marker (100bp DNA Ladder : Invitrogen)

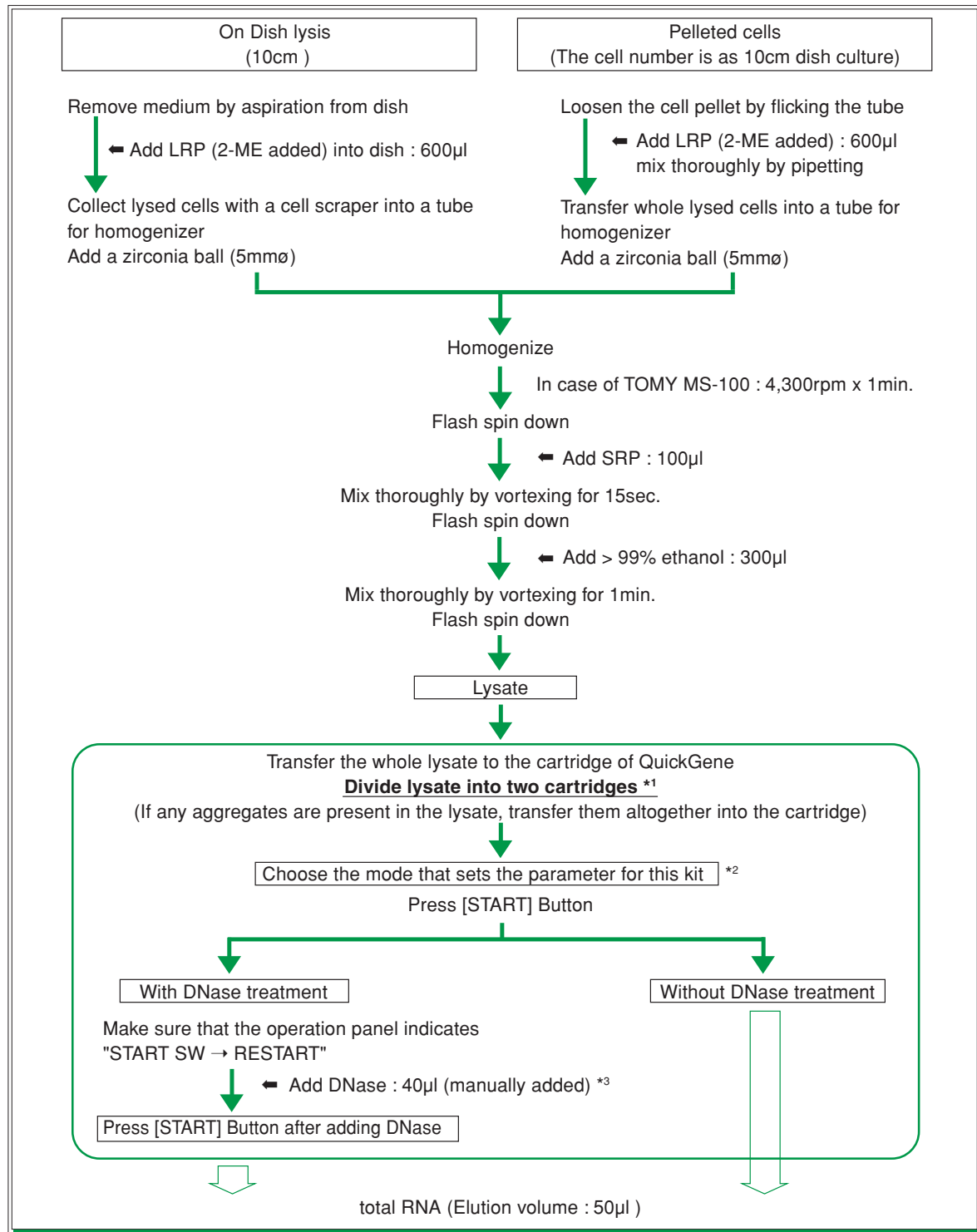
1 : QuickGene

2 : Spin column method(A company)

N : Negative control

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

Protocol B



*1 : Two cartridges are used for one sample.

*2 : Regarding parameter setting method, refer to Kit Handbook appendix 1(p.24).

*3 : Please use recommended DNase products. As to the recommended products and solution preparation method, refer to Kit Hand Book.

Example of total RNA isolation from various cultured cells

Protocol B

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

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

● The yield of total RNA

Cell Line	Number of cells (x 10 ⁶ cells)	Yield (μg)			
		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	129.0	115.7	122.0	104.0
HEK293	5.0 - 8.0	175.3	92.2	160.3	101.0
COS-7	2.5	104.2	98.2	90.0	79.0
NIH / 3T3	4.5	89.4	100.2	79.0	84.0
HL60	15.0	167.3	154.4	144.4	140.5

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

● The purity of total RNA

Cell Line	Number of cells (x 10 ⁶ cells)	A _{260/280}				A _{260/230}			
		DNase(+)		DNase(-)		DNase(+)		DNase(-)	
		QuickGene	Spin column method	QuickGene	Spin column method	QuickGene	Spin column method	QuickGene	Spin column method
HeLa	5.0	2.20	1.99	2.20	2.02	2.18	2.10	2.05	2.12
HEK293	5.0 - 8.0	2.29	2.11	2.27	2.11	2.12	2.16	2.11	2.18
COS-7	2.5	2.12	1.97	2.12	2.05	2.11	2.03	1.94	2.19
NIH / 3T3	4.5	2.19	2.02	2.17	2.12	2.02	2.26	1.94	1.75
HL60	15.0	1.92	1.85	2.18	2.09	2.17	2.15	2.18	2.12

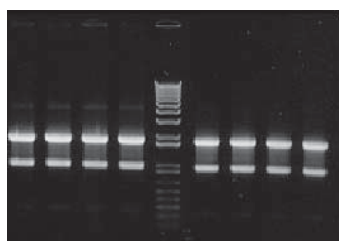
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

● Electrophoresis of total RNA (with DNase Treatment)

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

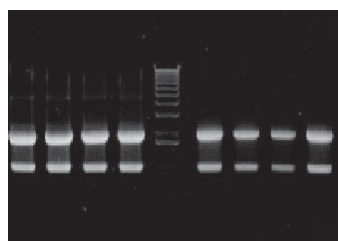
HeLa (10cm dish)

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



HEK293 (10cm dish)

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

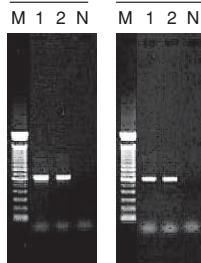


M : Marker
(1Kb Plus DNA Ladder : Invitrogen)

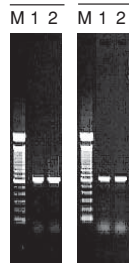
● RT-PCR

RT-PCR was performed with β-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 10⁶cells)
10pg/μl 1pg/μl



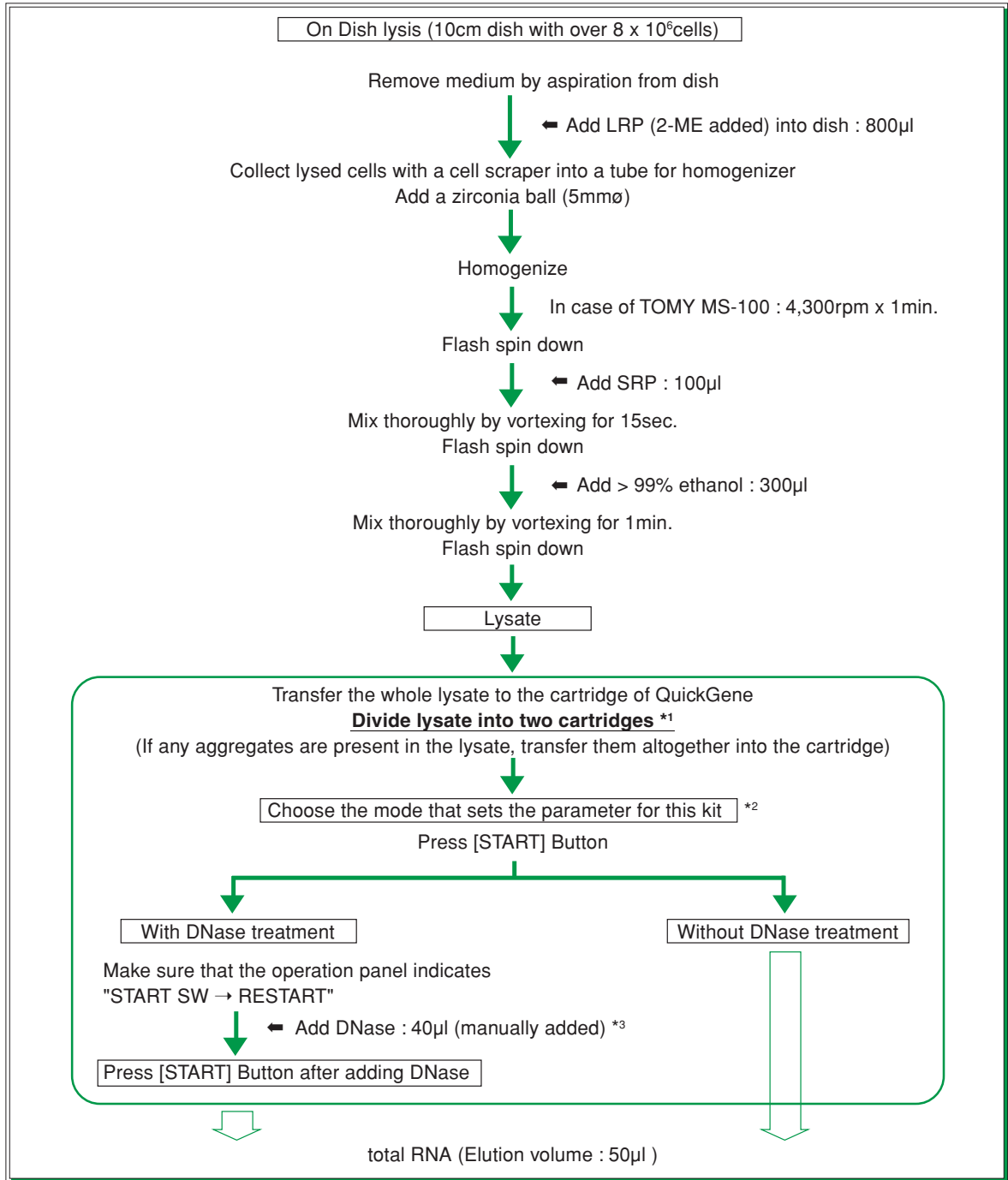
HeLa (10cm dish)
10pg/μl 1pg/μl



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.

Protocol B'



*1 : Two cartridges are used for one sample.

*2 : Regarding parameter setting method, refer to Kit Handbook appendix 1(p.24).

*3 : Please use recommended DNase products. As to the recommended products and solution preparation method, refer to Kit Hand Book.

Example of total RNA isolation from cultured cells

Protocol B'

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

Cell Line	Number of cells (x 10 ⁶ cells)	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.

● The purity of total RNA

Cell Line	Number of cells (x 10 ⁶ cells)	A _{260/280}				A _{260/230}			
		DNase(+)		DNase(-)		DNase(+)		DNase(-)	
		QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)	QuickGene	Spin column method (A company)
HEK293	12.0	1.95	2.04	1.98	2.02	2.14	2.14	1.88	2.17

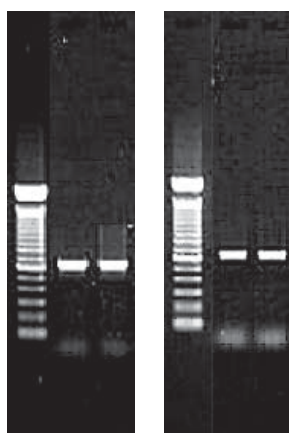
Use of QuickGene system enables high-purity total RNA isolation with little contamination of protein and chaotropic salt.

● RT-PCR (with DNase treatment)

RT-PCR was performed with β-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 10⁶cells)

10pg/μl			1pg/μl		
M	1	2	M	1	2



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.

Regarding homogenization

Homogenizer Model : Ball Mill Homogenizer

TOMY MS-100

Recommended tube : 2ml tube (Tomy Medico : Cat.No. 72693)

Ball : Zirconia 5mmø (Cat. No. ZB-50)

Homogenization condition

6cm dish : 2,800rpm x 2min.

10cm dish : 4,300rpm x 1min.



Topics Homogenizing effect of Ball Mill Homogenizer

Total RNA was isolated from 15×10^6 cells of HL60 using QuickGene system (QuickGene and QuickGene RNA cultured cell HC kit S) and Spin column method(A company).

Isolation condition for QuickGene system

Homogenizer : TOMY MS-100

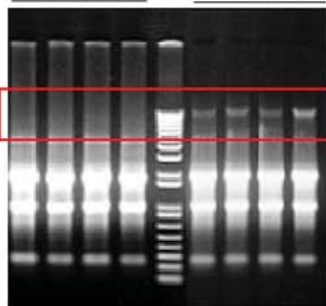
Homogenization condition : 4,300rpm x 1min.

Protocol : B

DNase treatment : no treatment

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

QuickGene M Spin column method



M : Marker (100bp DNA Ladder : Invitrogen)

In Spin column method(A company) where homogenization treatment with ball mill homogenizer is not performed for lysate, contamination of genomic DNA is detected. On the other hand, in QuickGene system where the treatment is performed, little contamination of genomic DNA is detected even without DNase treatment because of DNA decomposition by homogenization.

* Trade mark and exclusion item

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North American Distributor

www.autogen.com

AutoGen, Inc. 84 October Hill Road Holliston, MA 01746

Tel: 508.429.5965; Fax: 508.429.9765; E-mail: info@autogen.com