Total RNA Extraction from Cultured Cells

Total RNA Extraction from Cells Cultured in 6cm, 10cm Dish

Kit : QuickGene SP kit RNA cultured cell HC (Spin method)

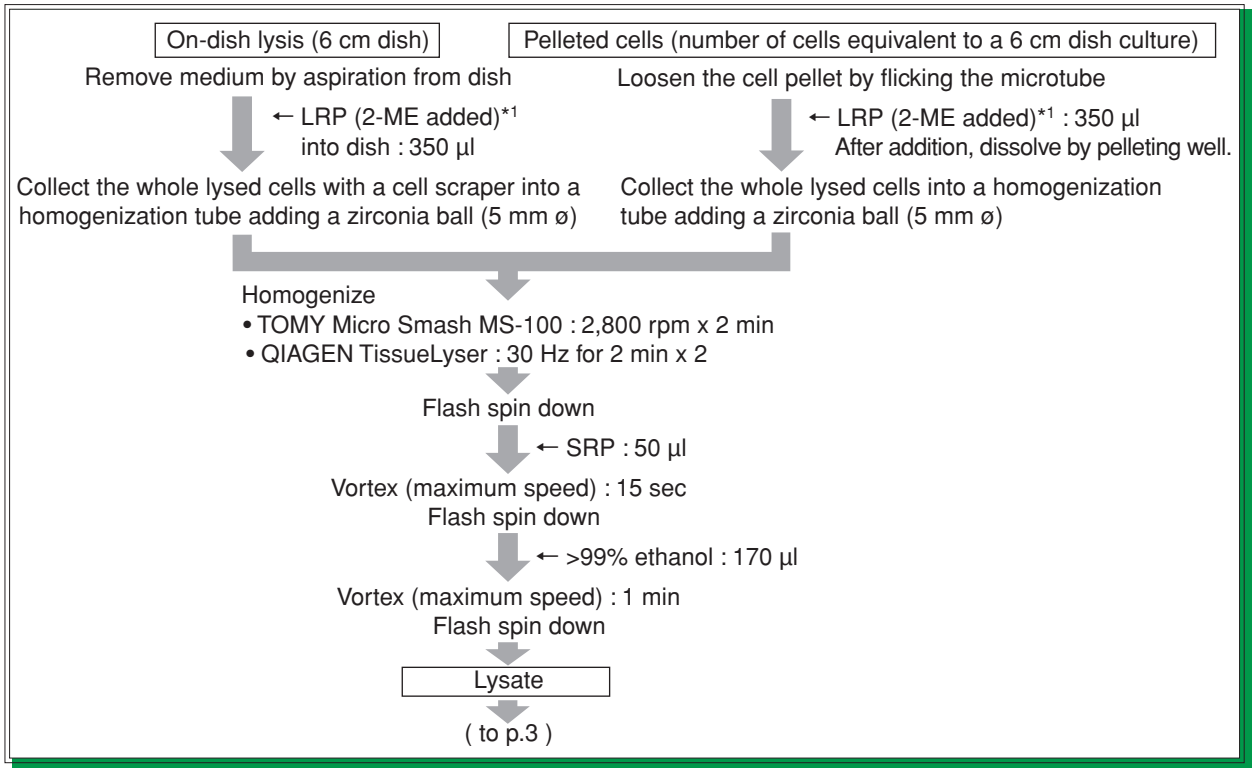
Protocol

< Number of cells and corresponding protocols >
Before performing an extraction, please select an extraction protocol by reference to following table.

Dish size	Cell species	Number of cells (x 10^6)	Protocol
6cm	HeLa	1.5~3.5	A
	HEK293	3.0~5.0	
	COS-7	0.5~1.5	
	NIH/3T3	1.0~2.5	
	HL60	3.0~5.0	
10cm	HeLa	3.5~5.5	B
	HEK293	5.0~8.0*	
	COS-7	2.0~3.0	
	NIH/3T3	3.0~5.0	
	HL60	5.0~15	
	HEK293	8.0~15*	B'

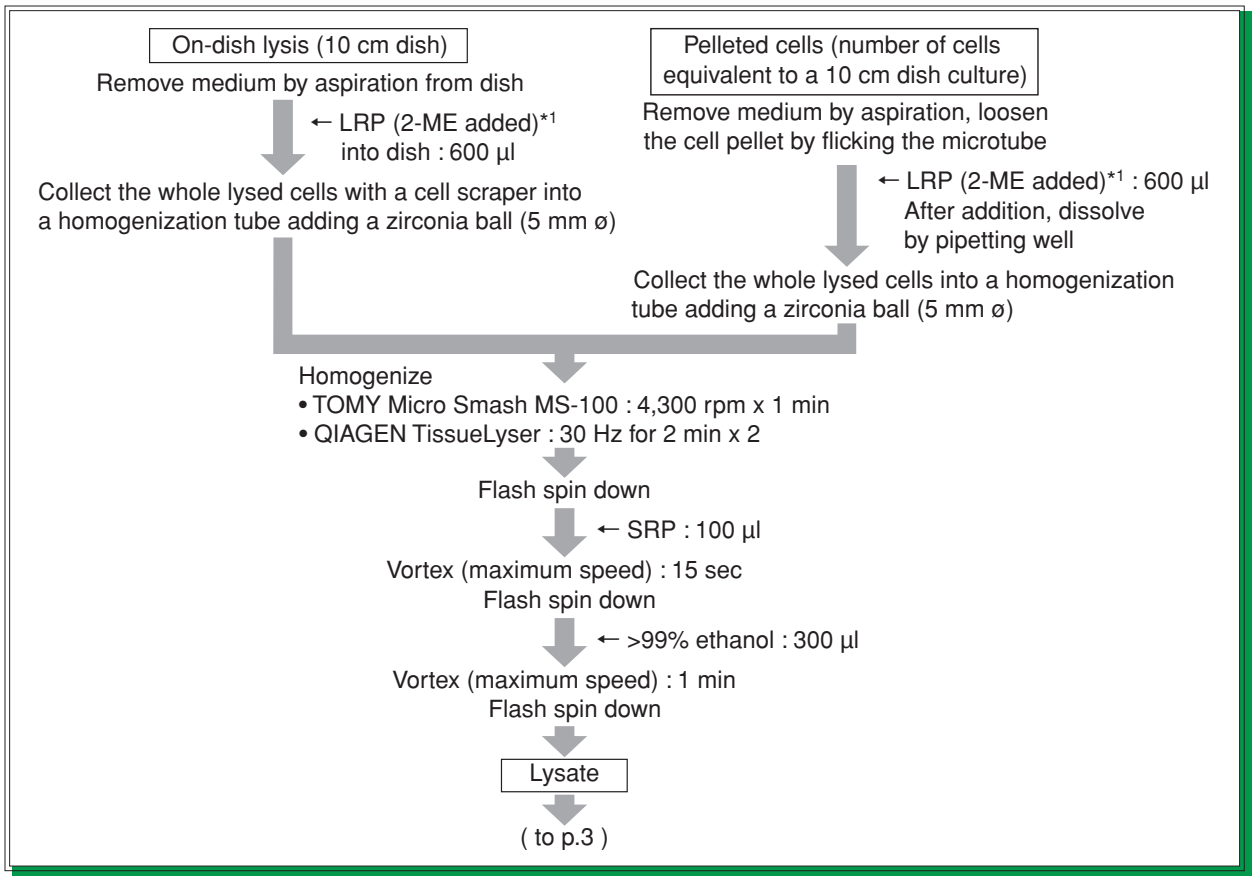
* If lysing a number of cells exceeding 8.0 x 10^6 directly on a dish, use Protocol B'.
Pelleted cells can be processed up to 15 x 10^6 with Protocol B.

● Protocol A



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

● Protocol B



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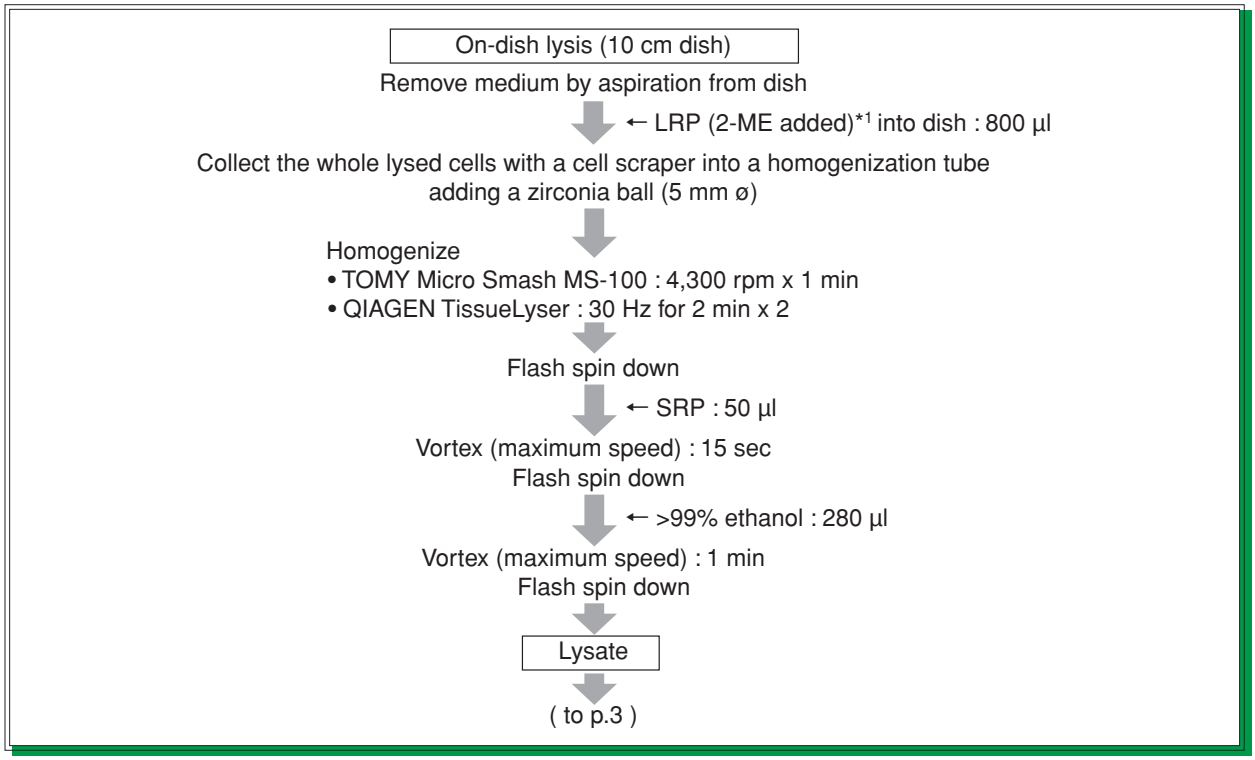
● Protocol B'



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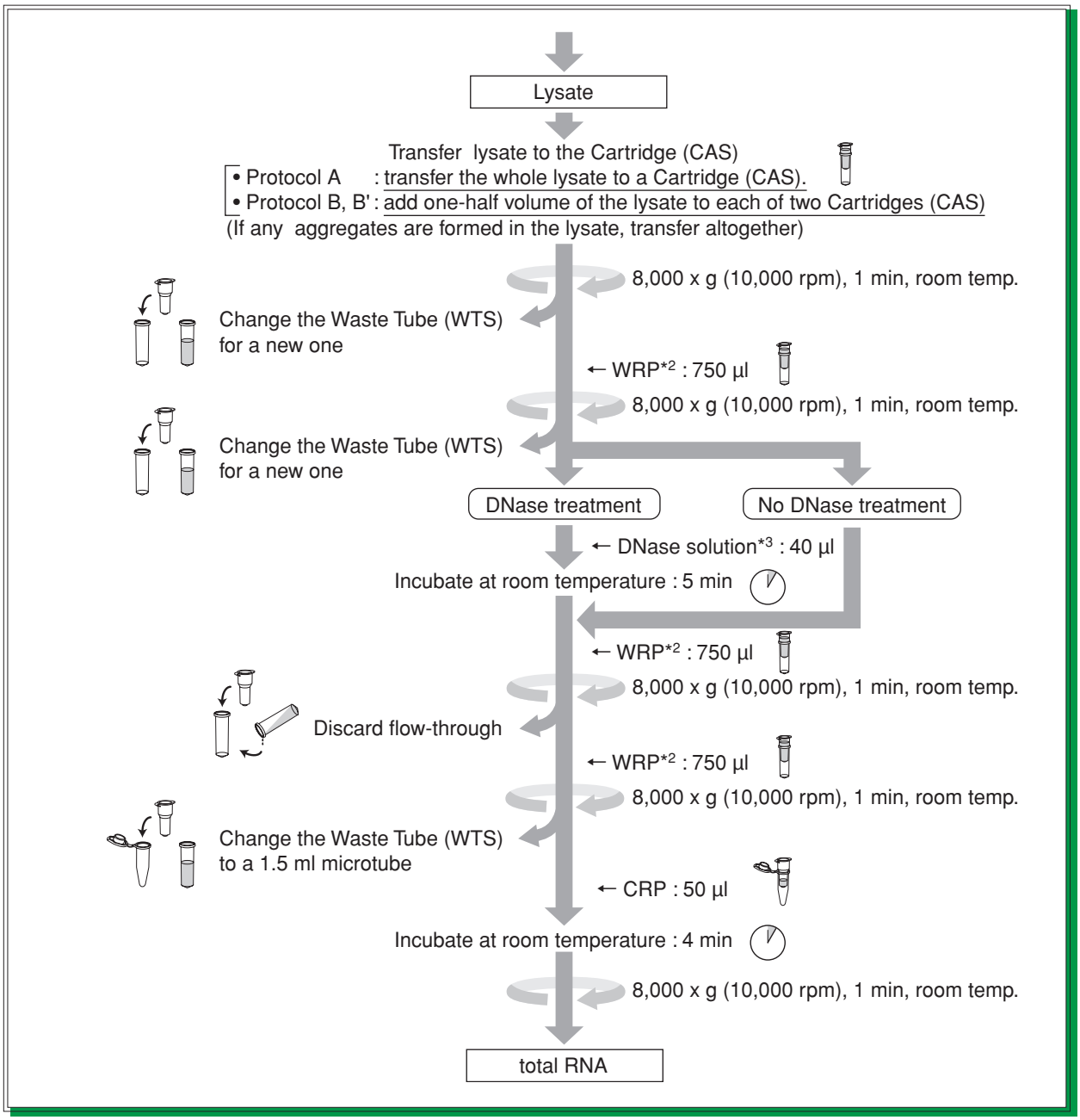


*2 : Add 25 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use.
*3 : DNase is not included in the kit. Please prepare following recommended product.

Recommended DNase

a) RQ1 RNase-Free DNase	(Promega	: Cat. No. M6101)
b) DNase I, Amplification Grade	(Invitrogen	: Cat. No. 18068-015)
c) DNase I, Amplification Grade	(SIGMA	: Cat. No. AMP-D1)
d) Deoxyribonuclease (RT Grade)	(Nippon Gene	: Cat. No. 313-03161)
e) DNase I, RNase-Free	(Ambion	: Cat. No. 2222)
f) RNase-Free DNase Set	(QIAGEN	: Cat. No. 79254)

In the case of DNase a)~d)

1 U / µl DNase I	: 20 µl
10 x Reaction Buffer	: 4 µl
Nuclease-free water	: 16 µl

In the case of DNase e)

2 U / µl DNase I	: 20 µl
10 x Reaction Buffer	: 4 µl
Nuclease-free water	: 16 µl

In the case of DNase f)

2.7 Kunitz unit / µl DNase I	: 1.25 µl
Buffer RDD	: 35 µl
Nuclease-free water	: 3.75 µl

QuickGene

Results : Total RNA extraction from various model cells

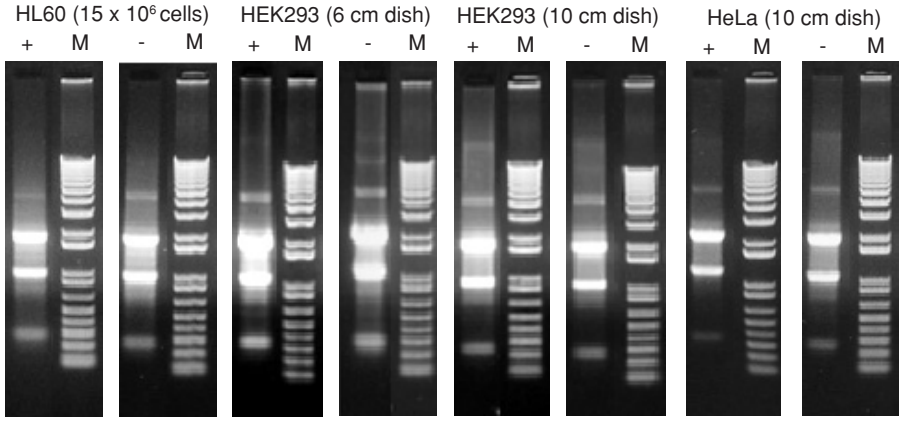
Total RNA was extracted from various model cells with QuickGene SP kit RNA cultured cell HC.

● The yield and purity of total RNA

Cells species		HL60		HEK293		HeLa		COS-7		NIH / 3T3	
Protocol		A	B	A	B'	A	B	A	B	A	B
Cell form		Pellet		6 cm dish	10 cm dish	6 cm dish	10 cm dish	6 cm dish	10 cm dish	6 cm dish	10 cm dish
Number of cells (x 10^6 cells)		5.0	15	4.5	9.9	1.7	4.9	1.0	2.7	1.7	4.5
DNase(+)	Yield(μg)	44.0	167.4	89.1	213.0	52.0	126.5	40.4	110.0	32.5	97.1
	$A_{260/280}$	2.17	2.15	2.18	2.16	2.17	2.19	2.21	2.21	2.23	2.20
	$A_{260/230}$	2.23	2.18	1.98	2.21	2.08	2.23	2.06	2.22	2.15	2.21
DNase(-)	Yield(μg)	47.7	165.7	84.0	224.2	-	127.7	-	-	-	-
	$A_{260/280}$	2.17	2.15	2.19	2.17	-	2.18	-	-	-	-
	$A_{260/230}$	2.22	2.21	2.01	2.21	-	2.13	-	-	-	-

● Electrophoresis of total RNA

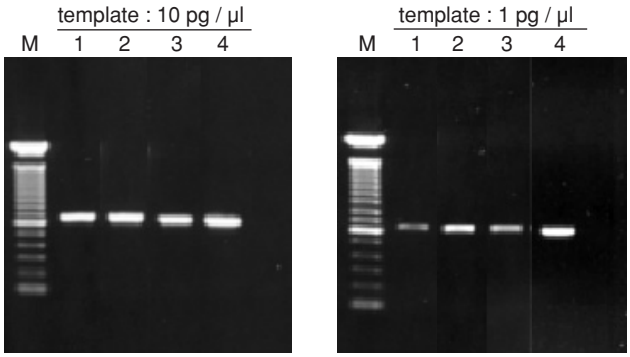
Electrophoresis conditions : Non denaturing gel electrophoresis (1% Agarose gel / 1 x TAE Buffer)



M : Marker
(1kb Plus DNA Ladder : Invitrogen)
\+ : DNase treatment
\- : No DNase treatment

● RT-PCR (with DNase treatment)

RT-PCR was performed with total RNA extracted from various sample cells using QuickGene SP kit RNA cultured cell HC.



Primer : β -actin
Electrophoresis conditions : 2% Agarose gel / 1 x TAE Buffer

M : Marker (100 bp DNA Ladder : Invitrogen)
1 : HL60 (15 x 10^6 cells)
2 : HEK293 (10 cm dish)
3 : HeLa (6 cm dish)
4 : Positive control
5 : Negative control

For RT-PCR performed on template 1 pg / μl, electrophoretic bands of amplification products were detected.

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FUJIFILM Corporation 7-3, Akasaka 9-Chome, Minato-ku, Tokyo 107-0052, Japan, Tel : +81-3-6271-2158, Fax : +81-3-6271-3136 • E-mail : sginfo@fujifilm.co.jp
FUJIFILM Europe GmbH Heesenstr.31, 40549 Dusseldorf, Germany, Tel:+49-211-5089-174, Fax:+49-211-5089-9144 • E-mail:lifescience@fujifilmeurope.de
FUJIFILM UK Ltd. Unit 12 St Martins Way, St Martins Business Centre, Bedford, MK42 OLF, U.K.,Tel:+44-1234-245291, Fax:+44-1234-245293 • E-mail:lifesciences@fuji.co.uk
富士胶片（中国）投资有限公司 31st floor, Hong Kong New World Tower, No.300 Huai Hai Zhong Road, Shanghai, P.R China, Tel:+86-21-3302-4655 ext.363, Fax:+86-21-6384-3322 • E-mail:wgxiang@fujifilm.com.cn
FUJIFILM Medical Systems U.S.A., Inc. Tel:+1-866-902-3854 Fax:+1-203-327-6485 • E-mail:don.wilke@fujimed.com
http://lifescience.fujifilm.com/

LS事-07・05（F283V）・050-1（F283V）