NIPPON Genetics EUROPE

Application

RNA extraction from cultured cells with the FastGene® RNA Premium Kit

(Supplement: Clean up using FastGene® RNA mini-elute column)

Product: FastGene® RNA Premium Kit（FG-81050, FG-81250）

Manufacturer: NIPPON Genetics EUROPE

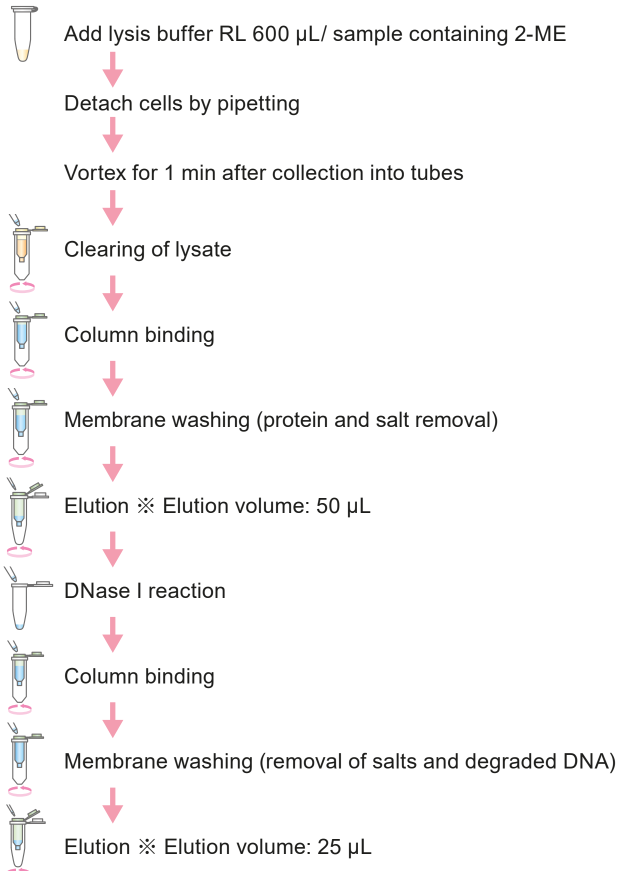
The following data has been published due to the kindness of domestic customers.

Points of this application note (NIPPON Genetics Co. Ltd)

The FastGene® RNA Kit is a RNA purifcation kit based on the silica membrane method, with "high quality at low costs". In the FastGene® RNA Premium kit, DNase treatment is performed after elution to ensure efficient removal of genomic DNA. We worked on improvement and stability.
This application note presents an example of using the FastGene® RNA Premium Kit.

Steps
- Used case of FastGene® RNA Kit (RNA extraction from cultured cells)
- Removal of genomic DNA by DNase treatment after elution

Workflow from RNA purification to real-time PCR



Sample preparation

Mouse primary cultured hepatocytes 1×10^{6} cell

RNA extraction

Add lysis buffer RL 600 µL/ sample containing 2-ME
↓
Detach cells by pipetting
↓
Vortex for 1 min after collection into tubes
↓
Clearing of lysate
↓
Column binding
↓
Membrane washing (protein and salt removal)
↓
Elution ※ Elution volume: 50 µL
↓
DNase I reaction
↓
Column binding
↓
Membrane washing (removal of salts and degraded DNA)
↓
Elution ※ Elution volume: 25 µL

Yield and purity —— Result 1

Measuring instrument: SimpliNano（GE）

Reverse transcription

Reagents: PrimeScript™ RT Master Mix（Perfect Real Time)（Takara)

●Reaction composition

5 x PrimeScript RT Master Mix	2 µL
total RNA	about 500 ng
RNase Free dH_2O	up to 10 µL
Total volume	10 µL

●Reaction program

37 ℃・15 min (reverse transcription reaction)
↓
85 ℃・5 sec (heat inactivation)
↓
4 ℃・hold

Real time PCR —— Result 2

Reagents: KAPA SYBR Fast qPCR kit（Universal qPCR kit)
（KAPABIOSYSTEMS)

Machine: Thermal Cycler Dice Real Time System II（Takara)

●Reaction composition

KAPA SYBR FAST qPCR Master Mix（2X)	10 µL
10 µM forward primer	0.4 µL
10 µM reverse primer	0.4 µL
Template DNA	equivalent to 10 ng
PCR-grade water	up to 20 µL
Total volume	20 µL

●Reaction program

95 ℃・3 min (initital denaturation)
↓
95 ℃・2 sec (denatured)
↓
60 ℃・30 sec (annealing/ extension reaction)

(95 ℃・2 sec → 60 ℃・30 sec: 40cycles)

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Result

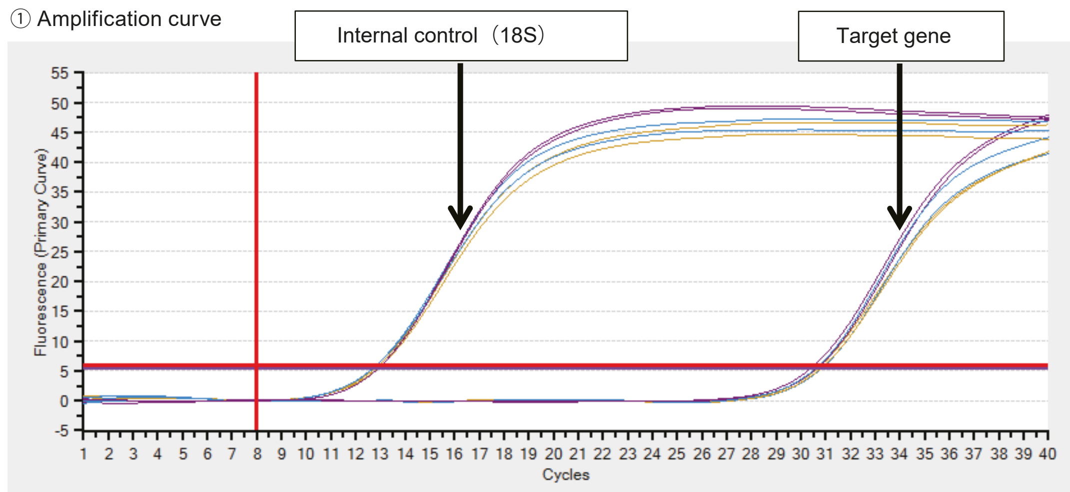
Result 1. Yield and purity measurement

SampleNo.	Conc. (ng/μL)	A_{260}/A_{280}	A_{260}/A_{230}	SampleNo.	Conc. (ng/μL)	A_{260}/A_{280}	A_{260}/A_{230}
1	59.2	2.0	1.9	13	48.9	2.0	1.8
2	33.2	2.0	2.0	14	45.6	2.0	1.8
3	42.3	2.0	1.9	15	20.5	1.9	1.5
4	41.8	2.0	1.8	16	55.7	2.0	1.7
5	43.4	1.9	1.7	17	41.5	2.0	1.7
6	53.3	2.0	1.8	18	32.0	2.0	1.7
7	59.6	2.0	1.7	19	27.6	1.9	1.5
8	54.5	2.0	1.9	20	36.7	2.0	1.9
9	47.0	2.0	1.9	21	29.1	1.9	1.4
10	43.5	2.0	1.9	22	19.5	2.0	1.8
11	50.0	2.0	1.9	23	58.9	2.0	2.0
12	63.3	2.0	2.0				

Result 2. Real time PCR

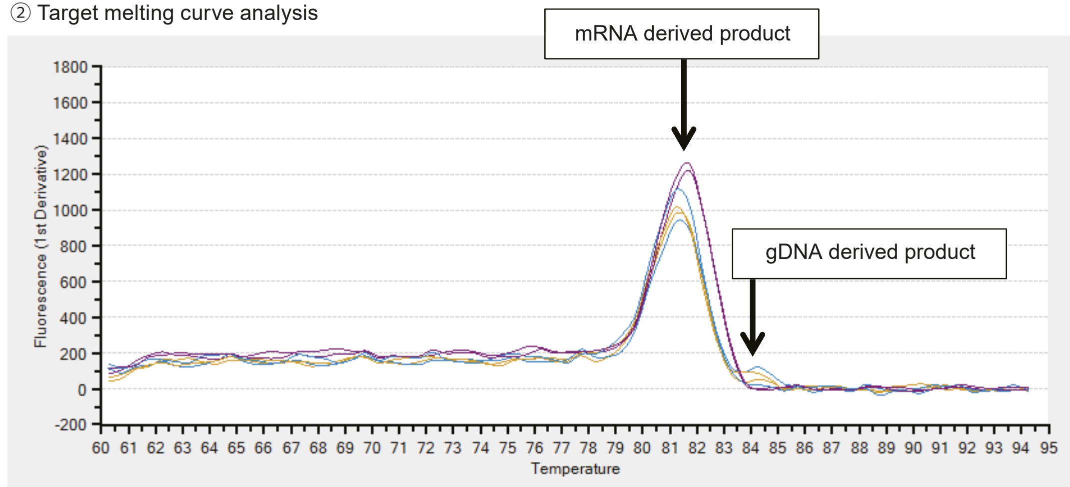
※ Among the results of the above yield and purity measurement, the results of representative samples are shown.

① Amplification curve



cDNA was prepared from RNA extracted with FastGene® RNA Premium Kit and subjected to RT PCR. Good amplification was observed for both internal control (18S ribosomal RNA) and target gene.

② Target melting curve analysis



Melting curve analysis confirmed specific amplification of mRNA derived products.
It is thought, that gDNA was effectively removed by FastGene® RNA Premium Kit.

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Supplement

◎Clean up of TRIzol extracted RNA with FastGene® RNA mini-elute column

●Procedure

Extracted RNA from cells using TRIzol —— Supplemental results (1)

Genomic DNA removal with DNase (Promega)

Inactivation of DNase and RNA reextraction with TRIzol (dissolution with about 50 µL of H_2O) —— Supplemental results (2)

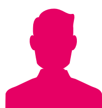
Add H_2O to the same volume (50 µL) after DNase treatment of FastGene® RNA Premium Kit

RNA extraction by protocol using FastGene® RNA mini-elute column

Absorbance measurement —— Supplemental result (3)

●Result

No.	(1) RNA extracted by TRIzol			(2) (1) DNase treatment of 5 µg RNA and purification with TRIzol			(3) (2) RNA mini-elute column		
	Concentration (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}	Concentration (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}	Concentration (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}
1	381.3	1.7	2.1	165.8	1.6	1.9	114.8	2.0	1.7
2	406.6	1.8	2.0	181.2	1.6	1.9	124.8	2.0	1.8
3	435.5	1.8	2.0	161.3	1.6	1.8	111.3	2.0	1.7
4	429.5	1.8	1.9	162.5	1.6	1.9	104.1	2.0	1.9
5	418.9	1.8	2.0	152.2	1.6	2.0	104.0	2.0	1.8
6	412.0	1.8	2.0	152.7	1.6	2.1	103.3	2.0	1.9
7	268.5	1.7	2.0	119.1	1.7	0.9	80.9	2.0	1.7



Customer's comment

In the previous RNA extraction method (phenol/chloroform extraction), the product derived from gDNA was amplified due to the contamination of gDNA. (It can not be avoided by primer design due to the structure of the gene).

Therefore, after phenol/chloroform extraction, DNase treatment was performed separately, concentration measurement and reverse transcription was performed again.

The old method took time and effort, but with this kit it was reduced greatly, which was extremly helpful. Analysis of the melting curve (Result 2-②) also revealed no gDNA-derived product and I was very satisfied with the result.

In addition, it is thankful that it contains all homogenization, purification and DNase treatment in one kit, which is also inexpensive.

I am relieved that there is a little surplus in the amount of lysis buffer and DNase.

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