





Comparative study of reverse transcriptase reaction using RNA extracted from peritoneal cells of sterile peritonitis model mouse

Product name

FastGene® Scriptase II (LS53, LS63)

Manufacturer

NIPPON Genetics EUROPE

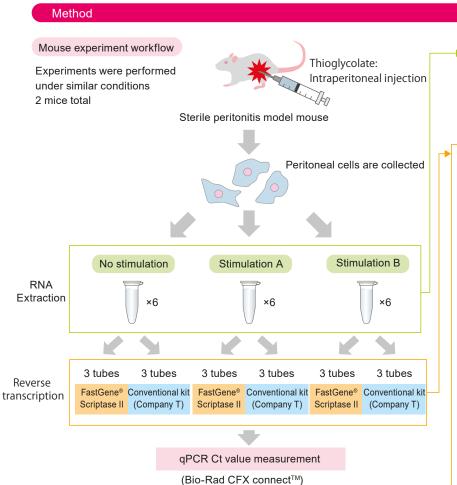
The following data has been posted due to the kindness of customers of the University of Tokyo, Japan.

Introduction

We have been conducting reverse transcriptase reactions using a conventional kit (T company) for a long time, but considering the use of this product in order to reduce costs and improve the accuracy of reverse transcripton reaction.

Using RNA extracted from peritoneal cells of aseptic peritonitis model mice, reverse transcription reaction was performed with this kit and the conventional kit (T company).

By performing qPCR with the obtained cDNA, the performance of the kit was compared by confirming the Ct value.



RNA extraction

Perform RNA extraction using TRIzol®

DNase treatment: None

RNA elution buffer volume: 100 µL DEPC water

RNA measurement: Thermo Fisher Scientific Nanodrop2000

Reverse transcriptase reaction (FastGene® Scriptase II)

- 1) Add 1 µL of Oligo dT primer and total RNA template of 100 ng
- 2) Add 2 µL dNTP
- 3) DW (distilled water) is added to a total volume of 12.5 μ L
- 4) Incubate at 65°C for 5 minutes and cool on ice immediately
- 5) Add components

5x FastGene® Scriptase II buffer	4	μL
0.1 M DTT	2	μL
RNase Inhibitor	0.5	μL

- 6) Incubate at 42°C for 2 minutes
- 7) To the RNA suspension on ice,
 1 μL FastGene[®] Scriptase II is added
- 8) Incubate at 42°C for 50 minutes
- 9) Incubate at 70°C for 15 minutes to completely inactivate the enzyme



SFast Gene[™] Scriptase II

- Obtain longer cDNA for low RNase H activity
- Optimized for qPCR recombinant enzyme

qPCR (KAPA SYBR Fast qPCR Kit)

 Reaction composition 		
Component	Volume	Final
KAPA SYBR Fast qPCR Master Mix (x2)	5 μL	1x
Forward Primer 10 μM	0.2 µL	200 nM
Reverse Primer 10 μM	0.2 µL	200 nM
Template DNA	as necessary	<20 ng
SDW Add to	make a total amount of	10 μL N/A

Cylce program			
Enzyme Activation	95℃	5 min	
.	0506	40 -	
Denaturation	95°C	10 sec	10
↓ Annealing/Extension	60°C	30 sec —	40 cycles



Result

Yield and purity measurement result of input RNA (extraction by TRIzol®)

mouse No.1

RNA amount (ng/μL) purity (A260/A280) No stimulation 11.2 1.89 Stimulation A 30.9 1.84 Stimulation B 7.8 1.85

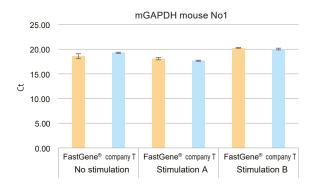
mouse No.2

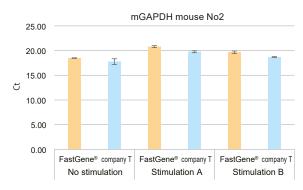
RNA	amount (ng/μL)	purity (A260/A280)
No stimulation	57.1	1.66
Stimulation A	9.8	1.91
Stimulation B	19.1	1.71

Comparison by qPCR

mGAPDH

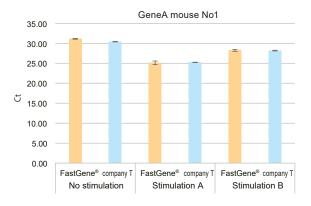
Gene	RT Kit		mouse	Ct	Mean	StdDev Ct
			No stimulation	18.50	18.58	0.51
				19.13		
			18.12			
				18.32		0.23
		No.1	Stimulation A	17.87	18.08	
			18.04		1	
			20.17			
			Stimulation B	20.32	20.24	0.07
	FastGene®			20.24		
	rasidene			18.42		0.06
			No stimulation	18.46	18.47	
				18.54	1	
				20.60		
		No.2	Stimulation A	20.78	20.80	0.20
			Junia augusti / t	21.00		
				19.79		0.26
			Stimulation B	19.35	19.64	
			Sumulation B	19.79		
mGAPDH				19.22	19.27	0.11
			No stimulation	19.18		
			NO Stillulation	19.40	19.27	
				17.76		
		No.1	Stimulation A	17.56	17.65	0.11
		140.1	Sumulation A	17.63	17.05	0.11
				20.02		
			Stimulation B	20.02	20.02	0.18
			Surriulation B		20.03	0.18
company T			19.85			
			N = -4:1-4*	17.40	17.76	0.59
			No stimulation	18.44		
			17.44			
		N 0		19.70	40.77	0.04
	No.2	Stimulation A	19.61	19.77	0.21	
				20.00		
				18.70	18.69	0.08
		Stimulation	Stimulation B	18.60		
				18.76		

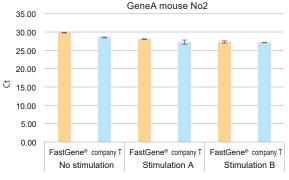




Gene A

Gene	RT Kit		mouse	Ct	Mean Ct	StdDev Ct
			30.99			
			No stimulation	31.18	31.12	0.11
			31.18			
			24.70			
		No.1	Stimulation A	25.04	25.11	0.45
				25.59		
			27.98			
			Stimulation B	28.23	28.23	0.25
	FastGene®			28.47		
	rasiGene			29.95		0.11
			No stimulation	29.81	29.83	
				29.74	20.00	
				28.19		
		No.2	Stimulation A	28.02	28.06	0.12
			Cumalation	27.97	20.00	0.12
				27.33	27.30	0.33
			Stimulation B	27.60		
		Ottilidiation D	26.95	21.50	0.55	
Gene A				30.35	30.40	0.06
			No stimulation	30.47		
			INO Stillidiation	30.40	30.40	
				25.22		
		No.1		25.22	0.06	
		140.1	Still diation A	25.28	25.22	0.00
				28.14		
					0.09	
			Garridiadori B	28.27	20.10	0.09
company T			28.41			
			No stimulation	28.50	28.52	0.12
		INO Sumulation	28.65	20.52	0.12	
			26.96		0.57	
	No.2	Stimulation A	26.86	27.24		
	140.2	Sumulation A		21.24		
			27.90			
		Stimulation B	Stimulation B	27.07	27.13	0.06
				27.13		
			27.18			











We conducted experiments using the conventional kit and FastGene® Scriptase II, but we have seen that the results of real-time PCR showed nearly equivalent results.

It was nearly the same for the trouble of operation.

The price tends to be lower in the FastGene $^{\scriptsize @}$ series, so I think that is the merit.