



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

Application

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 transcriptase 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.

## Method

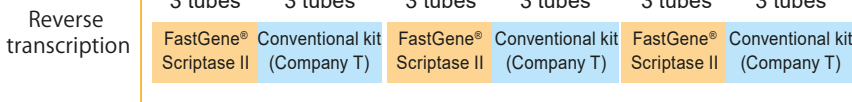
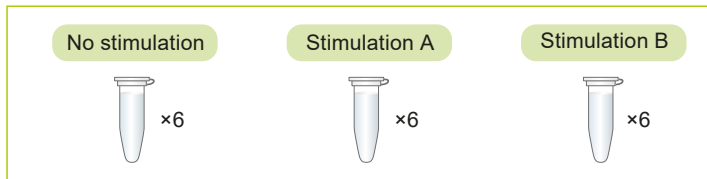
### Mouse experiment workflow

Experiments were performed under similar conditions  
2 mice total



Sterile peritonitis model mouse

Peritoneal cells are collected



qPCR Ct value measurement  
(Bio-Rad CFX connect™)

### 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 µL
- 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



**FastGene® 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

#### ● Cycle program

Enzyme Activation	95°C	5 min	} 40 cycles
↓			
Denaturation	95°C	10 sec	
↓			} 40 cycles
Annealing/Extension	60°C	30 sec	



### 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

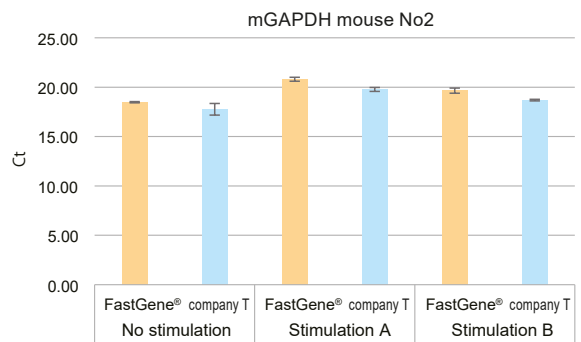
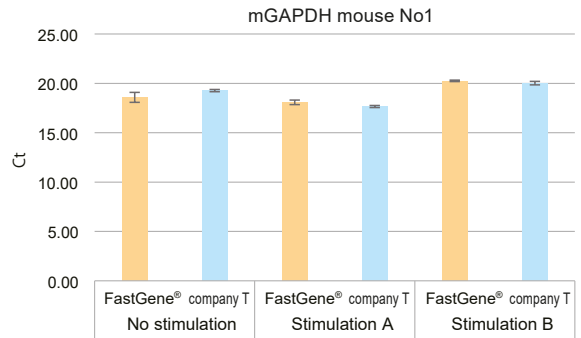
mouse No.2

	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

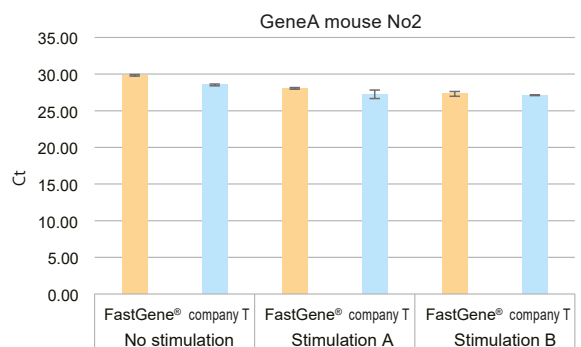
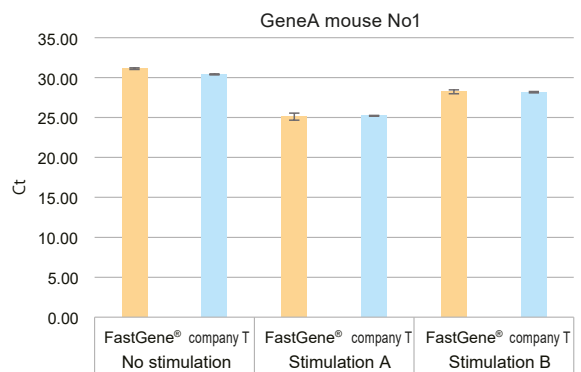
mGAPDH

Gene	RT Kit	mouse	Ct	Mean	StdDev Ct	
mGAPDH	FastGene®	No.1	No stimulation	18.50	18.58	0.51
				19.13		
				18.12		
			Stimulation A	18.32	18.08	0.23
				17.87		
				18.04		
		Stimulation B	20.17	20.24	0.07	
			20.32			
			20.24			
		No.2	No stimulation	18.42	18.47	0.06
				18.46		
				18.54		
	Stimulation A		20.60	20.80	0.20	
			20.78			
			21.00			
	Stimulation B	19.79	19.64	0.26		
		19.35				
		19.79				
	company T	No.1	No stimulation	19.22	19.27	0.11
				19.18		
				19.40		
			Stimulation A	17.76	17.65	0.11
				17.56		
				17.63		
Stimulation B		20.02	20.03	0.18		
		20.21				
		19.85				
No.2		No stimulation	17.40	17.76	0.59	
			18.44			
			17.44			
	Stimulation A	19.70	19.77	0.21		
		19.61				
		20.00				
Stimulation B	18.70	18.69	0.08			
	18.60					
	18.76					



Gene A

Gene	RT Kit	mouse	Ct	Mean Ct	StdDev Ct	
Gene A	FastGene®	No.1	No stimulation	30.99	31.12	0.11
				31.18		
				31.18		
			Stimulation A	24.70	25.11	0.45
				25.04		
				25.59		
		Stimulation B	27.98	28.23	0.25	
			28.23			
			28.47			
		No.2	No stimulation	29.95	29.83	0.11
				29.81		
				29.74		
	Stimulation A		28.19	28.06	0.12	
			28.02			
			27.97			
	Stimulation B	27.33	27.30	0.33		
		27.60				
		26.95				
	company T	No.1	No stimulation	30.35	30.40	0.06
				30.47		
				30.40		
			Stimulation A	25.22	25.22	0.06
				25.17		
				25.28		
Stimulation B		28.14	28.18	0.09		
		28.11				
		28.27				
No.2		No stimulation	28.41	28.52	0.12	
			28.50			
			28.65			
	Stimulation A	26.96	27.24	0.57		
		26.86				
		27.90				
Stimulation B	27.07	27.13	0.06			
	27.13					
	27.18					





Customer comment

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® series, so I think that is the merit.