



# 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 transcription reaction.

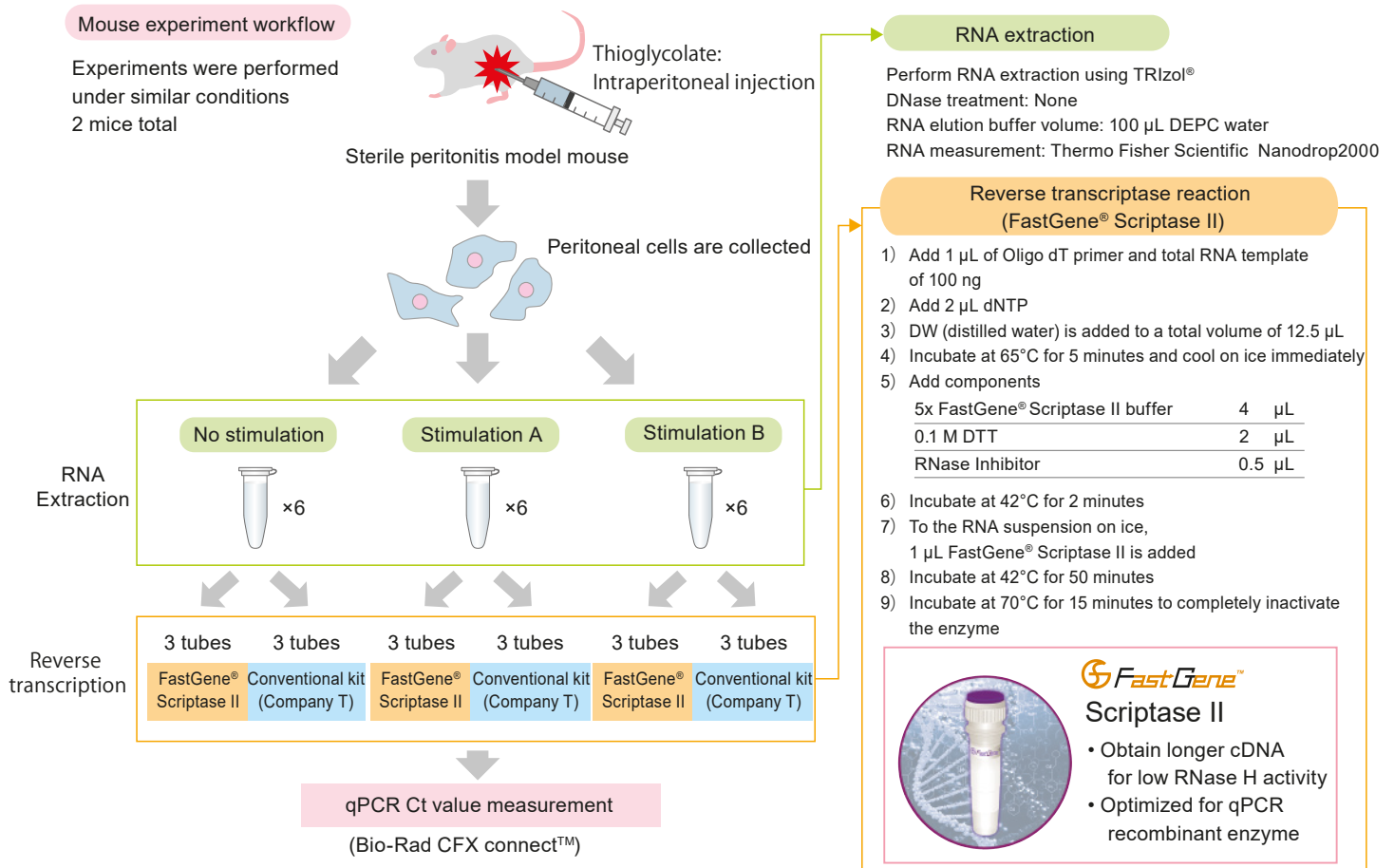
Using RNA extracted from peritoneal cells of aseptic peritonitis model mice, reverse transcription reaction was performed with this kit and the conventional kit (T company).

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



### 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

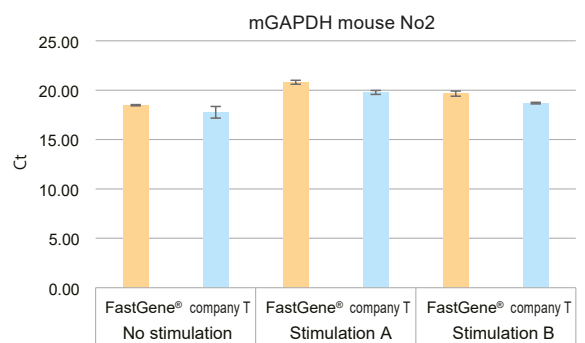
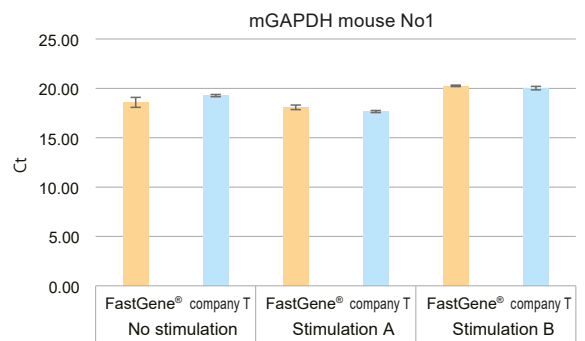
mouse No.2

	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

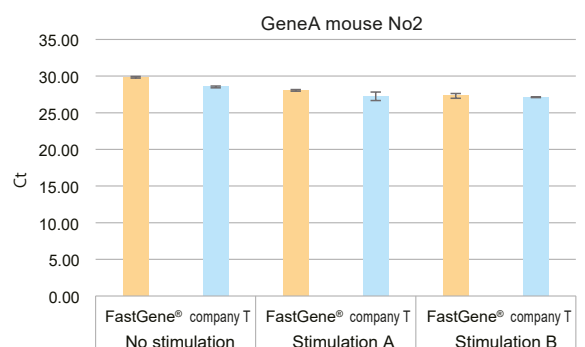
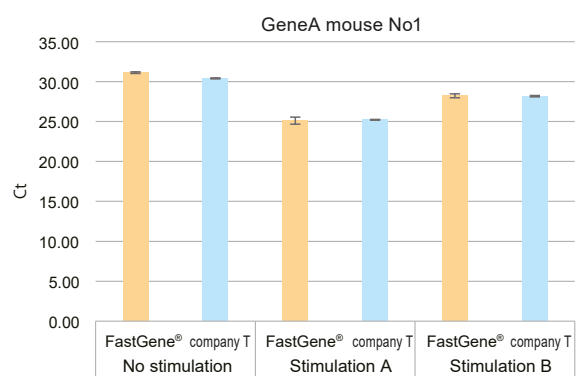
mGAPDH

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					
				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	No stimulation	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.

