NIPPON Genetics EUROPE

Technical Data

FastGene® 0.1 mL PCR tube evaluation test

Product

FastGene® 0.1 mL 8-well PCR strips（Cat.No.FG-017FC）
FastGene® 0.1 mL single PCR tube（Cat.No.FG-011F）
FastGene® 0.1 mL 8-well PCR strips with flat caps（Cat.No.FG-018WF）

Purpose

Evaluate the performance of FastGene® 0.1 mL PCR tube in real time PCR.

Evaluation

FastGene® 0.1 mL PCR tubes shape evaluation, evaporation test and a real time PCR test was performed.
In addition, from the analysis of the amplification and calibration curve, the influence of the cap on the reuslts of real-time PCR due to the difference in transparency etc. was evaluated.

Evaluation test
1. Tube shape evaluation　2. Evaporation　3. Real time PCR test

Evaluation product

① FastGene® 0.1 mL 8-well PCR strips
(Cat.No.FG-017FC)
(FastGene® CapEasy v2 compatible)

② FastGene® 0.1 mL single PCR tube
(Cat.No.FG-011F)

③ FastGene® 0.1 mL 8-well PCR strips with flat caps
(Cat.No.FG-018WF)

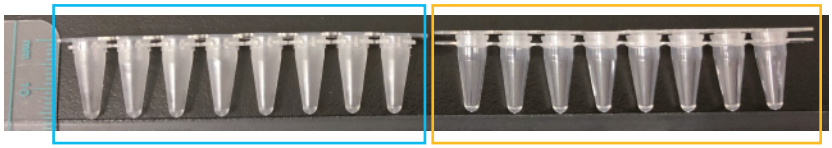
1. Tube shape evaluation

We evaluated whether the shape of each tube can be used for real time PCR equipment without any problems.

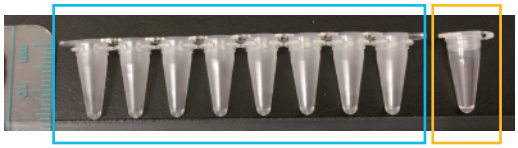
- Equipment used: Real-time PCR equipment（StepOnePlus™（Thermo Fisher Scientific））
- Control tube and cap:　Thermo Fisher Scientific MicroAmp™ Fast 8-Tube Strip, 0.1 mL（Cat.No.4358293）
  Thermo Fisher Scientific MicroAmp™ Optical 8-Cap Strips（Cat.No.4323032）

Difference in shape of each tube

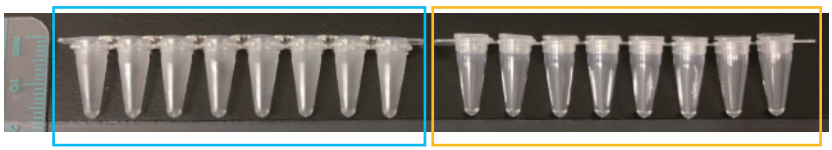
Control tube　① FastGene® 0.1 mL 8-well PCR strips



Control tube　② FastGene® 0.1 mL single PCR tube

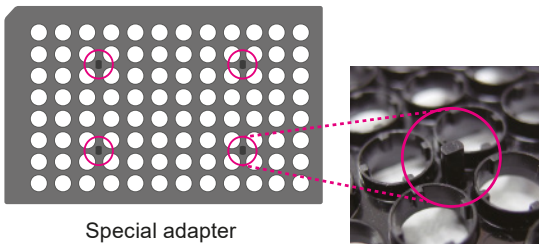


Control tube　③ FastGene® 0.1 mL 8-well PCR strips with flat caps



Tube and dedicated adapter

There were four protrusions on the dedicated adapter for the equipment used this time, and it was analyzed whether it could be set in the equipment, because there was a posibility that the tube would hit this protrusion.



Special adapter

①FastGene® 0.1 mL 8-well PCR strips and ②FastGene® 0.1 mL single PCR tube could be set in the device without hitting the protrusion of the dedicated adapter.

③FastGene® 0.1 mL 8-well PCR strips with flat caps hits the protrusion of the dedicated adapter, the connection between the tube and the lid was slightly bent, but the opening and closing of the tube was not affected, and there was no breackage and no liquid leakage.

2. Evaporation test

Runs were performed according to a program assuming a PCR test using each tube into which dyed water was dispensed. Changes in the amount of liquid due to evaporation were investigated.

Test procedure

1) To each tube 20 µL of dyed water was dispensed using a multichanel pipette and each cap was closed.
2) Spin down by using a centrifuge and visually check up.
3) Tubes were set on a dedicated adapter.

Tube arrangement

In order to see how pressure is applied to the tube and plate, it was arranged as follows.

Pattern A: minimum 3 rows

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Pattern B: maximum 12 rows

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

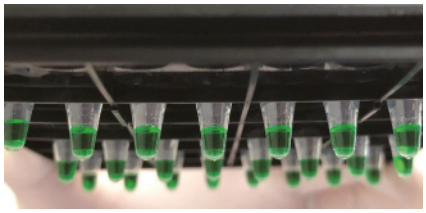
4) Set tubes in the real-time PCR equipment and conduct a run with the following program.

Program

Step	Temp	Time	Cycle
Initial denaturation	95℃	20 sec	
Cycle Reaction	95℃	3 sec	40 cycles
	60℃	30 sec	
Melting Curve	95℃	15 sec	
	60℃	1 min	
	95℃	15 sec	

5) After the run was completed, tubes were spun down and the presence or absence of evaporation was visually checked.

Evaporation test results



The run started without problems for all tubes and it ended successfully. There was no significant evaporation after the run.

3. Real time PCR test

We confirmed the effect of each tube itself on the results of real-time PCR test.

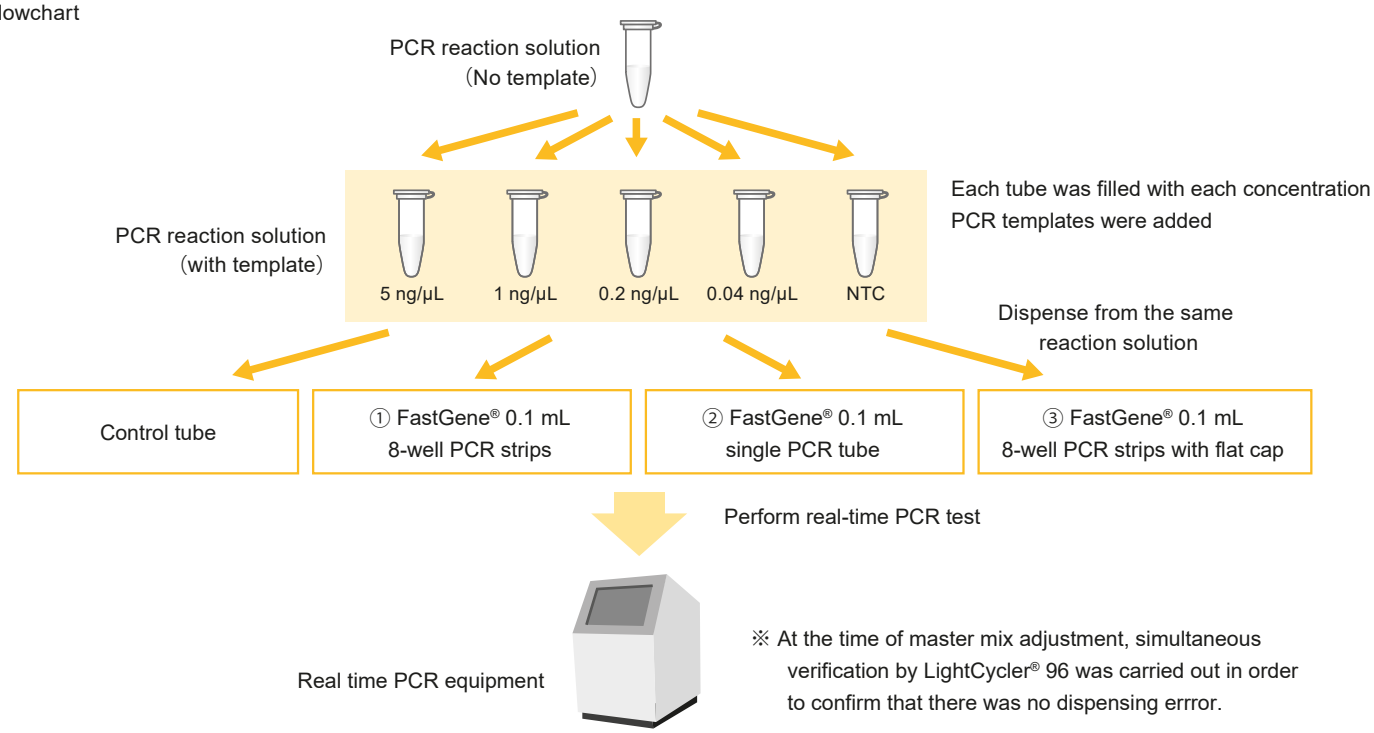
Test conditions

- Reagents used : KAPA SYBR Fast qPCR kit (Universal qPCR kit) (Cat.No. KK4601)
- Primer : Act-F1, Act-R1 (10 μM) <beta-actin: 294bp amplicon>
  Act-F1 : TCACCCACACTGTGCCCATCTACGA
  Act-R1 : CAGCGGAACCGCTCATTGCCAATGG
- PCR template : Roche Human Genomic DNA (Cat.No. 11691112001)
  Dilution series
  a. 5 ng/μL (1666.5 copies/μL)
  b. 1 ng/μL
  c. 0.2 ng/μL
  d. 0.04 ng/μL
  e. 0 ng/μL (Non Template Control: NTC)

- Reaction composition

Distilled water	7.6 μL
KAPA SYBER FAST qPCR Mster Mix (2×)	10.0 μL
PrimerF : Act-F1 (10 μM each)	0.2 μL
PrimerR : Act-R1 (10 μM each)	0.2 μL
Human Genomic DNA (a ～ e)	2.0 μL
Total	20.0 μL

- Flowchart



※ At the time of master mix adjustment, simultaneous verification by LightCycler® 96 was carried out in order to confirm that there was no dispensing errror.

Test procedure

1) A PCR reaction solution (without template) was prepared.
2) The PCR reaction solution (with no template) was dispensed into 5 bottles and templates with respective concentrations were added to prepare a PCR reaction solution (with template).
3) The PCR reaction soution (with template) of each concentration was dispensed into each of the following tubes (three each).

Tubes:
- Control tube
① FastGene® 0.1 mL 8-Tube PCR strips
② FastGene® 0.1 mL Single PCR tube
③ FastGene® 0.1 mL 8-Tube PCR strips with flat caps

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A			a			a				a		
B			b			b				b		
C			c			c				c		
D			d			d				d		
E			e			e				e		
F												
G												
H												

a. 5 ng/μL (1666.5 copies/μL)
b. 1 ng/μL
c. 0.2 ng/μL
d. 0.04 ng/μL
e. 0 ng/μL (Non Template Control: NTC)

4) Set in the real-time PCR machine and started the run.

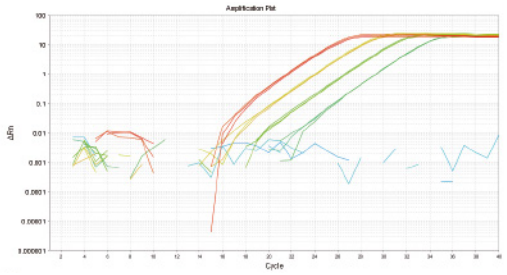
Program

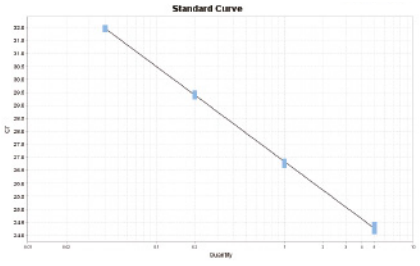
Step	Temp	Time	Cycle
Initial denaturation	95℃	20 sec	
Cycle Reaction	95℃	3 sec	40 cycles
	60℃	30 sec	
Melting Curve	95℃	15 sec	
	60℃	1 min	
	95℃	15 sec	

5) After the run, analysis was carried out, amplification curve, calibration curve and Ct value were confirmed.

Result of amplfication and calibration curve

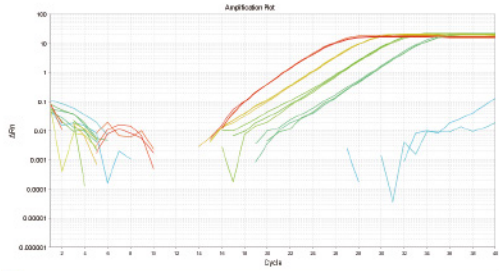
• Control tube

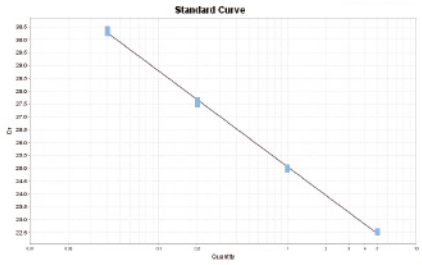




Eff% 87.035
R^2 0.999

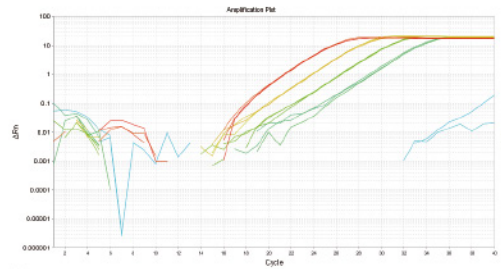
① FastGene® 0.1 mL 8-well PCR strips

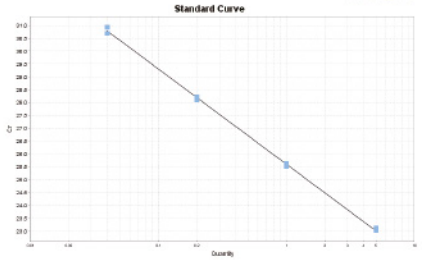




Eff% 85.724
R^2 0.998

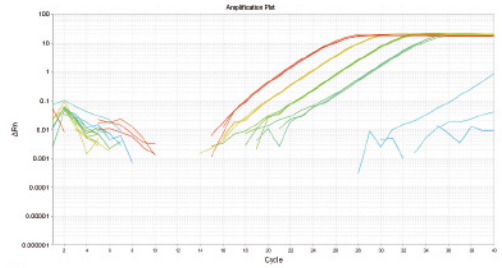
② FastGene® 0.1 mL single PCR tube

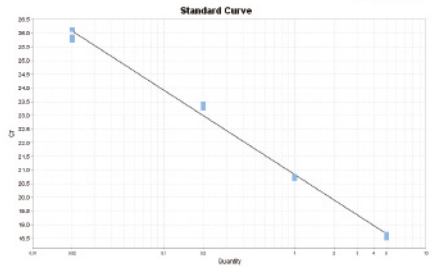




Eff% 85.994
R^2 0.999

③ FastGene® 0.1 mL 8-well PCR strips with flat cap





Eff% 110.711
R^2 0.994

The run ended successfully for all tubes.
An amplification curve consistent with the dilution series and a calibration curve with linearity (R^2 ≥ 0.99) were obtained.
(※ There was no problem checking PCR reaction solution by LightCycler® 96. Data not shown.)

Ct value result

The Ct value (Mean) was calculated for each tube and summarized below.

Tube \ PCR template	a. 5 ng/µL	b. 1 ng/µL	c. 0.2 ng/µL	d. 0.04 ng/µL
Control tube	24.29	26.8	29.43	31.97
①FastGene® 0.1 mL 8-well PCR strips	22.54	24.99	27.57	30.35
②FastGene® 0.1 mL single PCR tube	18.61	20.72	23.33	25.92
③FastGene® 0.1 mL 8-well PCR strips with flat cap	23.07	25.56	28.16	30.85

※ Each run was carried out for each tube.
This result is not the composition result between runs (it is between tubes).

Ct values consistent with diluton series were obtained for all tubes.

Summary of results

1. Tube shape evaluation
All of the evaluated tubes could be set in the used real time PCR equipment.

2. Evaporation test
The run started without problems for all tubes and ended successfully. No noticeable evaporation after visual run was observed.

3. Real time PCR test
From the data results, it was confirmed that PCR reaction was performed without problems.
Verification was done in 3 iterations and reproducibility was also obtained.

Summary

It was judged that the following FastGene® 0.1 mL PCR tubes could be used without problems for real-time PCR.

① FastGene® 0.1 mL 8-well PCR strips（Cat.No.FG-017FC）
② FastGene® 0.1 mL single PCR tube（Cat.No.FG-011F）
③ FastGene® 0.1 mL 8-well PCR strips with flat caps（Cat.No.FG-018WF）

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