NIPPON Genetics EUROPE

Application

Comparative evaluation of FastGene® Scriptase II and other company´s kit by RT-PCR

Category: Reverse transcriptase

Product: FastGene® Scriptase II（LS53）

Manufacturer: NIPPON Genetics EUROPE

The following data has been published du to the kindness of Mr. Tamura, a member of the Japanese public research institute.

Overview

FastGene® Scriptase II (LS53)

In the order to evaluate the performance of FastGene® Scriptase II, reverse transcription was performed on the RNA extracted from HeLa cells with this product and the other company´s reverse transcriptase.
The obtained cDNA was amplified by PCR for the target gene and confirmed by electrophoresis. The performance of FastGene® Scriptase II was evaluated by comparing the band signals.

Experiment

● RNA extraction
Sample: HeLa cells were cultured on 35 mm dish and used at 80-90% confluent time point.
RNA extraction: Ribozol (MS Technosystems) (N580-100ML)
Absorbance measurement: U-3900 (HITACHI) (U-3900)
Result: A260/280 = 1.72, yield = 41.5 μg

● Reverse transcriptase reaction
Total RNA was diluted to 1, 10, 100 ng/μL. 1 μL of each dilution was added as Template RNA.　※ 0 ng is 1 μL H_2O

● Primer
Gene specific primer (GAPDH)：CTCTTCCTCTTGTGCTCTTGC

FastGene® Scriptase II

Template RNA (0, 1, 10, 100 ng/μL)	： 1 μL
Gene specific primer (GAPDH)	： 2 μL (2 pmol)
dNTP Mixture (2 mM each)	： 2 μL
Sterile water (RNase free)	： 7.5 μL
total	: 12.5 μL

↓
65 ℃ 5 min
↓
4 ℃ 5 min
↓
add

5X FastGene® Scriptase II buffer	: 4 μL
0.1 M DTT (included in kit)	: 2 μL
FastGene® Scriptase II	: 1 μL

↓
42 ℃ 50 min
↓
70 ℃ 15 min
↓
keep at 22 ℃

Reference: Template total RNA recommended amount: 1 ng-5 μg

company T, RT reaction kit

Template RNA (0, 1, 10, 100 ng/μL)	： 1 μL
Gene specific primer (GAPDH)	： 2 μL (2 pmol)
2 mM dNTP	： 5 μL
RNase free dH_2O	： 2 μL
total	: 10 μL

↓
65 ℃ 5 min
↓
4 ℃ 5 min
↓
add

5X buffer	: 4 μL
Reverse transcriptase	: 1 μL
RNase free dH_2O	: 5 μL

↓
42 ℃ 50 min
↓
70 ℃ 15 min
↓
keep at 22 ℃

Reference: Template total RNA recommended amount: 5 μg or less

PCR conditions

● Reaction composition

10 μM Forward Primer	:	2.5	μL
10 μM Reverse Primer	:	2.5	μL
2 mM dNTPs (TOYOBO)	:	5	μL
5X Phusion HF Buffer	:	10	μL
cDNA	:	2	μL
sterile water	:	27.5	μL
Phusion DNA Polymerase (NEB)	:	0.5	μL
total	:	50	μL

● Program

95 ℃ 2 min
↓
95 ℃ 10 sec
65 ℃ 30 sec
72 ℃ 30 sec
] 30 cycles
↓
72 ℃ 3 min
↓
hold 22 ℃

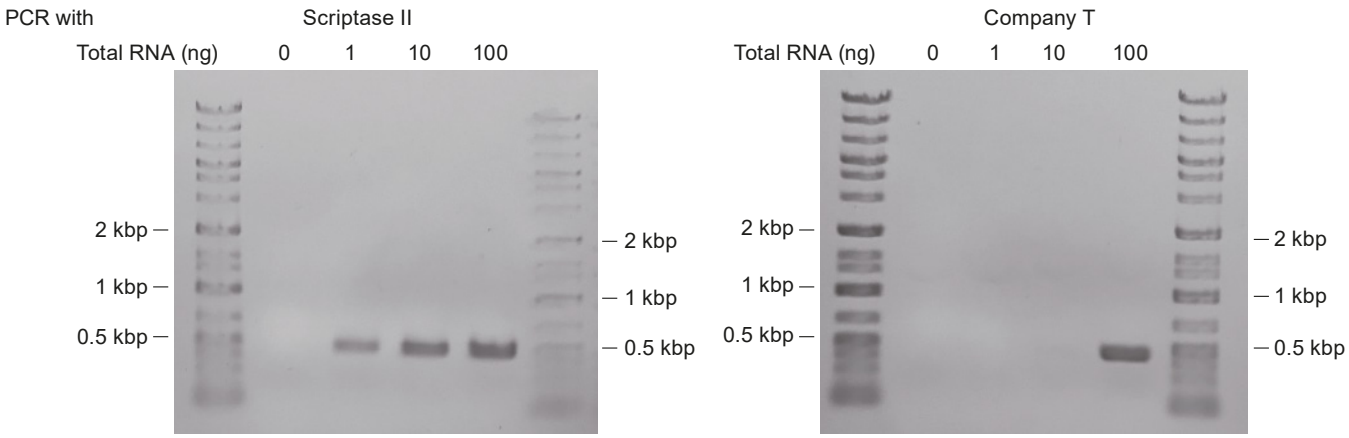
● Primers for-GAPDH

Forward Primer：CCACAGTCCATGCCATCAC
Reverse Primer：CCATGAGGTCCACCACCC
Amplification product size: 500bp

Electrophoresis condition

Agarose gel: 1% agarose gel
Sample: PCR reaction solution (15 μL)
Running buffer: 1xTAE
Migration conditions: 100 V, 30 min
Marker: 7 μL (Gene Ladder Wide 1) (Nippon Gene) (313-06961)

Result



※ 0 ng is 1 μL H_2O

We were able to detect PCR bands by using Scriptase II and a small amount of total RNA.

Customer`s comment

In order to analyze the mRNA expression level of cultured cells, I was searching for a reverse transcriptase that is convenient and can analyze a large amount of samples.
I am satisfied with reverse transcription with a small amount of total RNA.

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