NIPPON Genetics EUROPE

Application

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

Category: Reverse Transcriptase

Product: FastGene® Scriptase Basic (LS52)

Manufacturer: NIPPON Genetics EUROPE

The following data was published due to the kindness of Mr. Tamura, a member of the Japanese public research institute.

Overview

FastGene® Scriptase Basic (LS52)

In order to evaluate the performance of FastGene® Scriptase Basic, reverse transcription was performed on the RNA extracted from HeLA cells with this product and 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 Basic was evaluated by comparing the band signals.

Result

● RNA extraction
Sample: HeLa cells were cultured on a 35 mm dish and used at 70-80% confluency.
RNA extraction: Ribozol (MS Technosystems) (N580-100ML)
Absorbance measurement: U-3900 (HITACHI) (U-3900)
Result: A260/280 = 1.56, yield = 9.5 µg

● Reverse transcription 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 Basic

Template RNA (0, 1, 10, 100 ng/µL)	: 1 µL
Gene specific primer (GAPDH)	: 2 µL (2 pmol)
↓	
42 ℃ 5 min	
↓	
add	
10X FastGene® Scriptase buffer	: 2 µL
dNTP Mixture (2 mM each)	: 2 µL
FastGene® Scriptase basic	: 1 µL
Sterile water (RNase free)	: 12 µL
total	: 20 µL
↓	
42 ℃ 60 min	
↓	
90 ℃ 5 min	
↓	
keep at 22 ℃	

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

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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 ℃ for 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 ℃

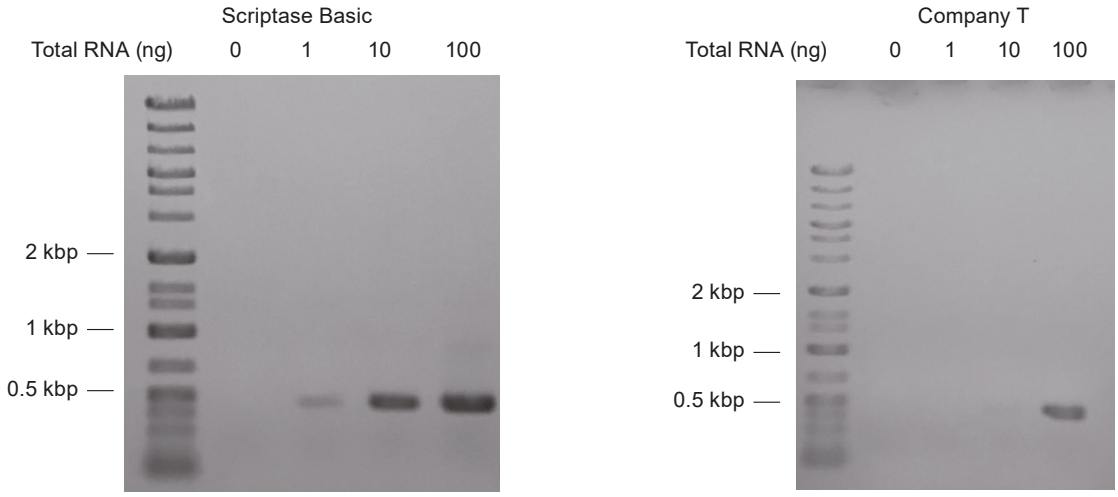
● Primers for-GAPDH

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) (Code No. 313-06961)

Result



※ 0 ng is1 μL H_2O

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

Customer's comment

Scriptase Basic is relatively inexpensive, so it is cost effective and satisfactory.
The tube labels are color-coded, which makes them easy to use.

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