NIPPON Genetics EUROPE

Application

Reverse transcriptase reaction for quantitative expression analysis using RNA extracted from mesenteric adipose rat tissue

Product category: Reverse transcriptase

Product: FastGene® Scriptase II Ready Mix (5X) (LS64)

Manufacturer: NIPPON Genetics EUROPE

The following data was kindly provided by Ms. Kazue Honma, Nutrition and Physiology Laboratory, Shizuoka Prefectual University, Japan

Features of FastGene® Scriptase II Ready Mix (5X) (LS64) (NIPPON Genetics EUROPE)

The reverse transcription kit used in this document is a Ready Mix type, designed for qRT-PCR for the purpose of quantifying gene expression, and includes random primers as reverse transcription primer.
Since quantification of gene expression by qRT-PCR often involves processing multiple samples, using a Ready Mix type can reduce the time required for dispensing operation.
In addition, since reverse transcription is performed using random primers, the heat denaturation step (65°C, 5 min) for solving the steric structure of RNA is omitted, and it is expected that target regions downstream of the steric structure are efficiently reverse transcribed.
By these, it is possible to simplify the operation and shorten the operation time in reverse transcription reaction.
Refer to “Points for selecting primer for reverse transcription” at the end of this document.

Background of examination

Expression analysis has been performed using RNA extracted from rat mesenteric adipose tissue.
The cDNA was synthesized from FastGene® Scriptase II cDNA Synthesis (LS63), but it felt a little time-consuming for the step of adding the enzyme and the step of solving the higher-order structure of RNA.
This time, using FastGene® Scriptase II Ready Mix (5X) (LS64) specialized for qPCR, the necessary reagents have already been mixed, and there are few operation steps, so it is easy to use.

Experiment

Untreated control rats and diabetic model rats treated with STZ(*) were used.

Rat → Mesenteric adipose tissue → Total RNA Extraction → RT reaction to cDNA → Relative quantitative analysis by qPCR

A: 8 Control animals
B: 7 diabetes models
12 days after administration of STZ
Blood sugar is around 590 at any times

※ STZ: Streptocin
Destruction of pancreatic islet β cells

RNA extraction:	QIAGEN RNeasy (74104)
RT reaction:	FastGene® ScriptaseII Ready Mix (5X) (LS64)
qPCR reagents:	SYBR Green I for LightCycler480 Master Mix (04707516001)
qPCR equipment:	LightCycler480 (Roche) (05015278001)

Reverse transcription reaction

FastGene® Scriptase II DNA Ready Mix(5x)

5x FastGene® Scriptase II Ready Mix	4	µL
High quality RNA-template	2～16	µL (400 ng)
Sterile Water	up to 20	µL
Total	20	µL

↓
25℃ 10 min：annealing step
↓
42℃ 60 min：Revese transcription reaction step
↓
85℃ 5 min ：Inactivation step of RT enzyme
↓
Store -20℃

Since the RNA concentration was different depending on the sample, it was normalized to 400 ng.

Experimental result

qPCR results

● Cq value

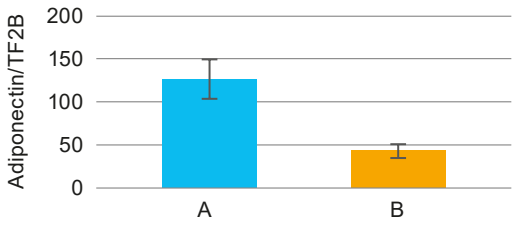
Targent gene: Adiponectin

Reference gene: TF2B

Rat		Cq value	
		Adiponectin	TF2B
Rat A Control	A1	26.01	33.87
	A2	23.63	30.80
	A3	24.76	29.95
	A4	29.38	36.80
	A5	27.62	34.04
	A6	26.16	31.90
	A7	23.70	30.69
	A8	23.41	30.76

Rat		Cq value	
		Adiponectin	TF2B
Rat B Diabetes mellitus model	B1	30.72	34.23
	B2	29.60	33.60
	B3	27.39	32.82
	B4	27.64	33.04
	B5	34.10	39.83
	B6	25.53	31.70
	B7	31.62	37.57

● Rel. quantitative result of Adiponectin



The mRNA expression of Adiponectin in mesenteric adipose tissue of the diabetic group was lower than in the control group.
Similar results are obtained with products that have been used so far.

Rat	Adiponectin / TF2B
A1	232
A2	144
A3	37
A4	171
A5	86
A6	53
A7	127
A8	163
AVE	126
SEM	23

Rat	Adiponectin / TF2B
B1	11
B2	16
B3	43
B4	42
B5	53
B6	72
B7	62
AVE	43
SEM	8

Customer's comment

The product is very easy to use, because everything was prepared in the mix.
In this case, the sample was incubated for 60 minutes, but since it has been shown that the reaction will be sufficient even for a short time, we expect to be able to reduce the working time further in future use.

Reference: Technical Note 2017〈EU1〉
Examination of ultrafast (~ 5 min) reverse transcription

Points for selecting primers for reverse transcription reaction

There are three types of appropriate primer for your downstream application. Primers used for reverse transcription are mainly random primers, oligo dT primers, target specifc primers (hereinafter specifc primers).

⇒ The product which is described in this document is a MasterMix, which contains random primers.

⇒ ScriptaseII is also available for selecting primers as Enzyme only (LS53) and cDNA synthesis kit (LS63).

(Features of each primer)

• Random primer:

Short primer with random base sequence. For example, in the case of random hexamers, 4096 types of primers are theoretically included. Since the RNA contained in the reaction solution can be comprehensively reverse transcribed from various positions, it is effective for the purpose of quantifying gene expression. In this case, prokariotic-derived mRNA that does not contain poly A tail or RNA having a secondary structure can be effectively reverse transcribed.

On the other hand, in eukaryote-derived mRNA, it is not suitable for cloning a full-length cDNA from the poly A tail side.

• oligo dT primers:

Primers annealing to poly A sequences. (Example: poly A tail of mRNA from eukaryote etc.)

Therefore, it is mainly used for full-length cDNA synthesis of mRNA derived from eukaryote.

In addition, in order to quantify the expression of eukaryotic genes, it can be expected to improve the reverse transcription efficency by using it as a mixture with random primers.

• Specific primers:

Primers designed based on the target RNA sequence.

It is used when detecting only a specific gene or when high specifity is required.

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