Cat. # 3789

For Research Use

TaKaRa

External Standard Kit (λ polyA) for qPCR

Product Manual

v201903Da

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I. Description

A reference gene is often used to normalize the total amount of input nucleic acid for gene expression analysis. The level of target gene expression is compared to the reference gene, a method called relative quantitation. Housekeeping genes are commonly used as the reference, but it may be difficult to identify a gene with stable expression in some experimental systems. In such cases, adding an external standard RNA to the reaction to serve as a reference may be useful for relative quantification.
This kit provides an external reference for real-time RT-PCR experiments. The kit contains a reference RNA (λ polyA$^+$ RNA-A), dilution solution (EASY Dilution), and a primer for detection (Real Time Primer for λ polyA). The RNA is an approximately 1.0 kb polyA-containing RNA synthesized by *in vitro* transcription using a λ DNA fragment template. This RNA can be used as an external reference in studies of eukaryotic gene expression by real-time RT-PCR. In addition, because the λ polyA$^+$ RNA-A contains a polyA sequence at the 3' end, reverse transcription reactions can only be primed with oligo dT.

II. Components

Real Time Primer for λ polyA	10 μM each	200 μl
EASY Dilution (for Real Time PCR)*		1 ml
λ polyA$^+$ RNA-A	10 ng/μl	15 μl

* EASY Dilution (for Real Time PCR) is available separately (Cat. #9160)

III. Storage

Real Time Primer for λ polyA and EASY Dilution (for Real Time PCR) : -20℃
λ polyA$^+$ RNA-A : -80℃

IV. Materials Required but not Provided

- Reverse transcription and real-time PCR reagents. For example:
  PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
  TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)*1
  One Step TB Green PrimeScript PLUS RT-PCR Kit (Perfect Real Time) (Cat. #RR096A/B)*1, 2
- 0.2 ml and 1.5 ml microtubes
- Micropipettes and tips
- Thermal cycler
- Real-time PCR instrument

*1 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series".
These products can be used the same way as before, as only the names are changing.
Catalog number and product performance are unaffected by this transition.

*2 Not available in all geographic locations. Check for availability in your area.

V. Protocol

1. Dilute λ polyA$^+$ RNA-A

Make serial dilutions of λ polyA$^+$ RNA-A 10 ng/μl (approximately 1.8 x 10^{10} copies/μl) with EASY Dilution (for Real Time PCR) to yield concentrations suitable for real-time RT-PCR experiments (see below and VI. Experimental Example). Aliquot in an appropriate volume, store at -80℃, and avoid repeated freeze-thaw cycles.

2. Add λ polyA$^+$ RNA-A to real-time RT-PCR reactions

The quantity of λ polyA$^+$ RNA-A that is added in the reaction varies depending on the step at which it is added. Adjust the quantity of λ polyA$^+$ RNA-A added so that the real-time PCR reaction mixture contains approximately 10^2 to 10^7 copies.

Examples:

When adding λ polyA$^+$ RNA-A to cell suspension before RNA extraction

Add 1.8 x 10^8 copies of λ polyA$^+$ RNA-A to 350 μl of cell suspension and make the final volume 50 μl after RNA extraction. In this case, the calculated concentration of λ polyA$^+$ RNA-A in the total RNA solution would be 3.6 x 10^6 copies/μl.

When adding λ polyA$^+$ RNA-A to RNA before reverse transcription

Add 1.8 x 10^7 copies of λ polyA$^+$ RNA-A to the total RNA to make a final volume of 10 μl. Use 2 μl of this mixture as the template for a reverse transcription (reaction volume, 20 μl). In this case, the concentration of λ polyA$^+$ RNA-A in the reverse transcription reaction would be 1.8 x 10^5 copies/μl.

VI. Experimental Example

Gene expression analysis with λ polyA+ RNA-A as reference (relative quantitation)

[Method]

1.8 x 10^8 copies of λ polyA+ RNA-A were added to 500 ng of each of four different total RNA samples from various mouse tissues. After reverse transcription by PrimeScript RT Reagent Kit (Perfect Real Time), λ polyA+ RNA-A and the target genes, Pah and Sord, were assayed by real-time PCR. Reverse transcription and PCR reactions were performed following the conditions provided in the product manuals. A standard curve was produced using samples from (a) liver as the standard.

Genes assayed : (A) λ polyA+ RNA-A; (B) Pah; (C) Sord

Sample : Total RNA from mouse (a) liver, (b) brain, (c) heart, and (d) kidney tissue
Experimental sample: cDNA, 1 ng each
Standard sample: liver cDNA, 5 pg to 50 ng, and no template control (NTC)
＊ The quantity of cDNA corresponds to total RNA-equivalent.

Reagent : PrimeScript RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B)

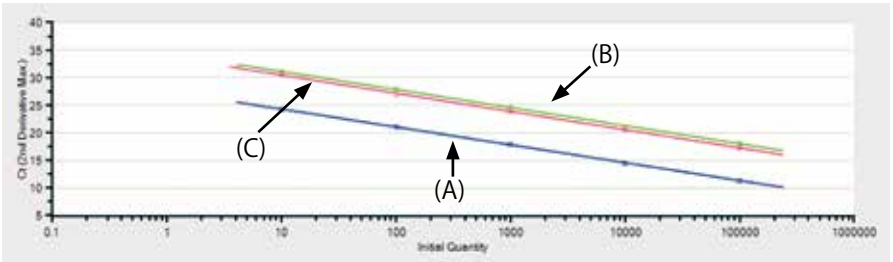
Apparatus : Thermal Cycler Dice™ Real Time System *III* (Cat. #TP900)＊

＊ Not available in all geographic locations. Check for availability in your area.

[Result]

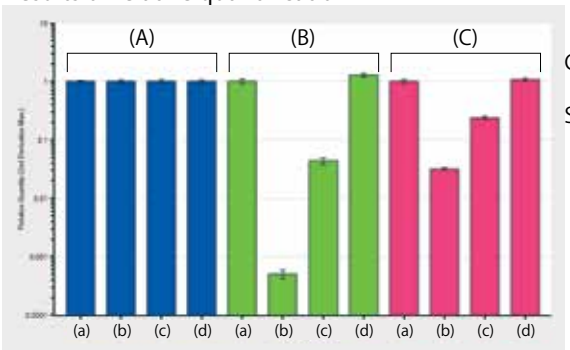
The standard curve created using the standard samples showed excellent accuracy for both the target genes and the external reference. The value for each experimental sample as determined from the standard curve was normalized to the λ polyA+ RNA-A reference, giving relative quantitation of Pah and Sord gene expression.

The standard curve for each gene



(A) λpolyA+ RNA-A (blue);	R2:1.000	Eff:103.6%	Y= -3.238 × LOG(X) + 27.52
(B) Pah (green);	R2:1.000	Eff:102.2%	Y= -3.271 × LOG(X) + 34.37
(C) Sord (pink) ;	R2:0.999	Eff:101.2%	Y= -3.294 × LOG(X) + 33.73

Results of relative quantification



Genes assayed:
(A) λ polyA+ RNA-A, (B) Pah, (C) Sord
Samples:
total RNAs from mouse tissues,
(a) liver, (b) brain, (c) heart, (d) kidney

VII. Reference

Gilsbach R, Kouta M, Bonisch H, and Bruss M. Comparison of *in vitro* and *in vivo* reference genes for internal standardization of real-time PCR data.
Biotechniques. (2006) **40**(2): 173-177.

VIII. Related Products

PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)
One Step TB Green® PrimeScript™ PLUS RT-PCR Kit (Perfect Real Time) (Cat. #RR096A/B)*
Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)*
Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)*
EASY Dilution (for Real Time PCR) (Cat. #9160)

* Not available in all geographic locations. Check for availability in your area.