ShinePolo® One Step RT-PCR Kits for qPCR User Manual

Cat.Nos.ZK00102(50 rxns×30ul)

ZK00103(100 rxns×30ul)

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Ⅰ、Description

ShinePolo® One Step RT-PCR Kits is a system for performing qRT-PCR in a single step, in a single tube. RNA does not work a template of PCR method. However, it becomes possible to apply the method to RNA analysis by synthesizing cDNA from RNA using reverse tramnscriptase. This is exactly the RT-PCR method. One Step RT-PCR is performed with this product.At One Step RT-PCR, a reverse transcription reaction performs using Specific Primer (Reverse).PCR performs using synthesized cDNA as a template, and using Specific primers (Forward,Reverse) as PCR primers. (Random Primer and Oligo dT Primer cannot be used for a reversetranscription reaction.) ShinePolo® is designed to allow real time RT-PCR reaction in one step with the use of SYBR Green I® or a detection probe, such as TaqMan®, Molecular beacon,etc.Ficoscript® is an engineered Moloney murine leukemia virus (MMLV) reverse transcriptase with reduced rNase H activity and optimized for use in a one step real-time system. PowerQ® Taq DNA Polymerase is a real-time quantitative PCR enzyme formulation consisting of recombinant full-length *Taq* polymerase (Engelke *et al*., 1990) and a highly efficient hot start antibody that inhibits *Taq* at ambient temperatures, providing automatic hot start PCR. The increased sensitivity and specificity exhibited by our PowerQ® formulation make it the perfect choice for your real-time quantitative PCR (qPCR) applications. One-step RT-PCR performs RT as well as PCR in a single buffer system. The reaction proceeds without the addition of reagents between the RT and PCR steps. This offers the convenience of a single-tube preparation for RT and PCR amplification. The operation is simple and it minimizes the risk of contamination. Amplified products are monitored in real time, so thers is no need to verify them through electrophoresis after PCR. This kit is suitable for detection of tiny amount of RNA like RNA virus. ShinePolo® is applicable to most real-time PCR instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, Roche,and Stratagene.

Ⅱ、Importants Notes

1. Multiple freeze/thaw of RNA should be avoided. Thaw and keep control RNA on ice.
2. It is recommended that the One Step RT-PCR is carried out under conditions where Rnase contamination has been eliminated. Pipette tips and tubes should be treated with 0.1% diethylpyrocarbonate (DEPC) (soak overnight in 0.1% aqueous solution of DEPC at 37℃, then heat at 100℃ for 30 min and autoclave).
3. Wearing gloves is highly recommended.
4. A separate RNA denaturation step is generally not required, but it can be performed before cDNA synthesis if the template RNA has a high degree of secondary structure. The denaturation step, 5 min at 65℃, should be performed before adding 2x RT buffer and RTase to the reaction mix.

Ⅲ、Component

Components	ZK00804 (50 rnsX 30ul each)	ZK00805 (100 rnx X 30ul each)
2×One Step Buffer	750ul	1500ul
RTase	100ul	200ul
DEPC-ddH2O	1ml	1ml

Ⅳ、Shipping and Storage

The ShinePolo® Kit is shipped in gel ice or ice bag. Upon arrival, store all kit components at -20°C. When using the kit, the leftover thawed mix can be refrozen and stored at -20 °C without affecting the performance of the kit. The kit is stable for six months from the date of packaging when stored and handled properly.

Ⅴ、Reaction Setup

Components	Volume(μl)	Comments
2× RT Buffer	15	Mix thoroughly
Sense primer(25pmol/ul)	1	Titrate from 0.3 to 1 μM if necessary.
Anti-sense primer(25pmol/ul)	1	Titrate from 0.3 to 1 μM if necessary.
Probe(25pmol/ul)orSybr Green I *	0.3	Titrate from 0.15 to 0.5 μM if necessary.
RTase	2	Mix thoroughly
RNA(ul)	X	It is recommended to use 10 pg-100 ng of total RNA as template.,usually 2-6ul
DEPC -ddH_2O (ul)	Y	Add water to fill up to the final reaction volume
Total Volume(ul)	20	

*SYBR Green I® should be diluted by 2,500-3,000 times with 10 mM Tris-HCl pH8.0 or dH2O, just before use. Then one-tenth volume of diluted SYBR Green I is added into the reaction mixture.

Ⅵ、Cycling Protocol

1、Protocol using LightCycler with Taqman probe

48℃20min→93℃ 2min→93℃5 sec→60℃30 sec
(40cyclers)

2、Protocol using LightCycler with Molecular Beacon probe or Sybr Green I

48℃20min→93℃ 2min→93℃5 sec→60℃20 sec→72℃20 sec

40cyclers

3、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Taqman probe

48℃20min→94℃ 4min→94℃ 30sec→60℃ 60sec

40cyclers

4、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Molecular Beacon probe or Sybr Green I

48℃20min→94℃ 4min→94℃ 30sec→60℃ 30sec→72℃30sec

40cyclers

Ⅶ、Reference

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2. Ausubel, F.M. et al. eds., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, 1997.
3. D'aquila, R. T., Bechtel, L. J., Videler, J. A., Eron, J. J., Gorczyca, P. & Kaplan, J. C. (1991) Maximizing sensitivity and specificity of PCR by preamplification heating. *Nucleic Acids Res.* **19**:3749.

4.Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(t)) Method.*Methods* 2001, 25:402-408.

5.L D Ke, Z Chen .A reliability test of standard-based quantitative PCR: exogenous vs endogenous standards Mol Cell Probes. 2000 Apr;14(2):127-35.

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Ⅷ、House-keeping genes

When studying gene expression, the quantity of the target gene transcript needs to be normalized against the quantity of a house-keeping gene transcript in the same sample. A house-keeping gene or several genes are used to normalize data against variation in sample quality and quantity between samples. Examples of commonly used house-keeping genes are GAPDH,beta actin and 18S rRNA. A gene used as a reference should have a constant expression level independent of the variation in the state of the

sample tissue. A problem is that even with housekeeping genes the expression usually varies to some extent. That is why several house-keeping genes are usually required, and their expression needs to be checked for each experiment.

Ⅸ、Relative quantification

Relative quantification is used to determine the ratio between the quantity of a target molecule in a sample and in the calibrator (calibrator being e.g. healthy tissue or untreated cells). The most common application of this method is the analysis of gene expression, e.g. comparisons of gene expression levels in different samples. Target molecule quantity is usually normalized with a house-keeping gene.Comparative C(t) method can be used for relative quantification. Both the sample and the calibrator data is first normalized against variation in sample quality and quantity. Normalized values, C(t)s, are first calculated from following equations:

$\Delta C(t)_{sample} = C(t)_{target} - C(t)_{house\text{-}keeping\ gene}$

$\Delta C(t)_{calibrator} = C(t)_{target} - C(t)_{house\text{-}keeping\ gene}$

The $\Delta\Delta C(t)$ is then determined using the following formula:

$\Delta\Delta C(t) = \Delta C(t)_{sample} - \Delta C(t)_{calibrator}$

Expression of the target gene normalized to the house-keeping gene and relative to the calibrator= $2^{-\Delta\Delta C(t)}$.

Ⅹ、Technical Supports

To access the ShineGene Web site, go to:**http://www.shinegene.org.cn**

The ShineGene Web site provides a list of telephone,Email and fax numbers that can be used to contact Technical Support.E-mail:master@shinegene.org.cn

Ⅺ、Related Products

ShinePrep® RNA Miniprep Kits

Cat.Nos.ZN00701(50 rxns)

ZK00702(100 rxns)

PowerQ® Taq plymerase

Cat.Nos.ZP00102(1000 U)

ZP00103(5000 U)

FicoScript® M MLV Reverse Transcriptase

Cat.Nos.ZP00601(1000 U)

ZP00602(5000 U)

ShineProbe® Real Time qPCR Kits

Cat.Nos.ZK00713(50 rxns×50ul)

ZK00714(100 rxns×50ul)

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