TIANGEN®

Quant One Step RT-qPCR Kit (SYBR Green)

For real-time RT-PCR using SYBR Green I

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Quant One Step RT-qPCR Kit (SYBR Green)

Cat. No. 4992885

Kit Contents

Kit Contents	4992885 50 µl × 50 rxn
2× Quant One Step RT-qPCR Mix(SYBR)[*1]	1.3 ml
Hotmaster Taq Polymerase (2.5 U/µl)	130 µl
Quant RTase (for one step)	30 µl
RNase-free ddH_2O	2 × 1 ml
Handbook	1

[*1] Containing dNTP Mixture, Mg^{2+}, SYBR Green I and ROX Reference Dye etc.

Storage

This kit (include ROX)should be stored at -20°C and protected from light.

Compatible Real-Time Instruments

ABI PRISM 7000/7700/7900HT, 7300/7500/7500 Fast Real-Time PCR System (Applied Biosystems)

DNA Engine OPTICON™/CFX96 (BIORAD)

Light Cycler480(Roche)

Smart Cycler® System (Cepheid)

Mx3000 P/ Mx3005 P(Stratagene)

Line-Gene(Bioer)

And others

Introduction

Quant One Step RT-qPCR Kit is designed for real-time one step RT-PCR by using SYBR Green I. The kit can be used in real-time one step RT-PCR of RNA targets. The reverse transcription and PCR can be carried out continuously in the same reaction tube, which is easy to operate and can minimize the risk of contamination. Also, Amplified products are monitoring in real time, so there is no need to verify them through electrophoresis after PCR. The kit is suitable for detection of tiny amount of RNA.

The proprietary reaction buffer has been specifically formulated to maximize activities of both Quant RTase and Hotmaster *Taq* DNA polymerase. Quant RTase is a new, unique enzyme, it exhibits a higher affinity for RNA, facilitating transcription through secondary structures that inhibit other reverse transcriptases, ensures highly efficient and sensitive transcription of RNA templates. The Hotmaster *Taq* DNA Polymerase formulation consists of a combination of TIANGEN's *Taq* Polymerase and the proprietary Hotmaster inhibitor. Inactive polymerase-inhibitor complexes are formed at temperatures below 40°C. When temperature is higher than 40°C, the Hotmaster inhibitor competes with the template DNA for binding to the Hotmaster *Taq* Polymerase, thereby shifting the binding equilibrium towards complex formation of template -specific primer. This hot-start procedure using Hotmaster *Taq* DNA Polymerase eliminates extension from nonspecifically annealed primers and primer-dimers in the first , which ensures highly specific and reproducible real-time PCR.

The kit uses a proprietary technology to provide accurate, sensitive and reproducible monoplex or multiplex RT-qPCR, provides a wide range of quantitative area for perfect standard curve and is compatible with a variety of real-time instruments.

Product Feature

1) The Real Time RT-qPCR can be finished in one step with quick and correct analysis of RNA virus and other tiny amount RNA.
2) Using the combination of improved Hotmaster *Taq* Polymerase .and specific buffer system researched by TIANGEN, Hot Start PCR can be processed with high amplification efficiency, sensitivity and specificity.
3) SYBR Green I and ROX Reference are pre-mixed in the kit, so the thawing step is simple and convenient.

Reagents and Materials not Supplied

1) Specific primers
2) Template RNA
3) Nuclease-free ddH_2O
4) Disposable gloves

Important Notes

1) Generate a scaled-up pre-mix containing PCR Master Mix (including enzymes, RNase-Free H_2O, buffer) and primers for the same gene, then aliquot into individual PCR tubes. In this way, the volume of the reagent can be more accurate, with less reagent loss and pipetting errors. In addition, it can reduce the error between the experimental operation and the experimental samples.
2) Gently mix Hotmaster Taq Polymerase and Quant RTase before use and centrifuge briefly prior to pipetting-avoid generating air bubbles. The enzyme is viscous due to the 50% concentration of glycerol, so pipet slowly. Store on ice all the time before use.
3) Shield the 2× Quant One Step RT-qPCR Mix(SYBR) from light.
4) Wear gloves to avoid RNase contamination from hands. Use RNase-free and filtered pipette tips.
5) Make sure the RT primer sequences are gene-specific. Do not use oligo(dT) or random primers.

Protocol

1) Prepare a reaction mix on ice according to the following table.

Components	Volume	Final Conc.
2× Quant One Step RT-qPCR Mix(SYBR)	25 μl	1×
Hotmaster *Taq* Polymerase (2.5 U/μl)	2.5 μl	
Quant RTase (for one step)	0.4 μl	
Forward primer	-	0.2 μM[*1]
Reverse primer	-	0.2 μM[*1]
Template RNA [*2]	-	-ng-pg
RNase-free ddH_2O	Up to 50 μl	-

[*1] A final primer concentration of 0.2 μM is optimal for most primer-template systems. However, in some cases using other primer concentrations (i.e., 0.1-1.0 μM) may improve amplification performance.

[*2] It is recommended to use 10 pg-100 ng total RNA as templates.

2) Set up the thermal cycler according to the procedure outlined in the following Table.

Centrifuge briefly before starting real-time PCR. For maximum yield and specificity, temperatures and cycling times can be further optimized for each new target and primer pair. However, the protocol gives satisfactory results in most cases.

Cycle numbers	Step	Temperature	Time	Comments
1×	1	50°C	30 min	Reverse transcription
1×	2	95°C	2 min	Initial PCR denaturation
35-45×	3	94°C	20 sec	Denaturation
	4	50-60°C	20 sec	Annealing
	5	68°C	20 sec	Extension

3) Result analysis

After the reaction is completed, validate melting curve and amplification curve. Establish the standard curve when quantitative analysis is necessary.