GoldStar Probe One Step RT-qPCR Kit
Project number: G665801

Storage condition: -20℃ keep away from light. If frequent use is required, store at 2-8℃ and try to avoid repeated freezing and thawing.

Product content

Component	G665801-100T
2×GoldStar Probe One Step Buffer	1.4ml
GoldStar Probe One Step EnzymeMix	100 μl
50 x High ROX	50 μl
RNase-Free Water	1.5ml

Product Introduction

This product is a specialized kit for one-step Real-Time RT-qPCR using the probe method (TaqMan, Molecular Beacon, etc.). When using this product for Real Time RT-qPCR reaction, reverse transcription and quantitative PCR are carried out in the same reaction system, and there is no need to add reagents or open the cap of the tube during the reaction process, which avoids contamination and improves the experimental efficiency at the same time. With high detection sensitivity, strong fluorescence signal and high signal-to-noise ratio, this product is very suitable for the detection of RNA viruses and other trace RNA. The special buffer system contained in this product can maximize the effectiveness of reverse transcriptase and DNA polymerase at the same time and improve the efficiency of the reaction. A wider linear range can be obtained with this product, more accurate quantification of the target gene, good reproducibility and high confidence.

ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, and other companies. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument.

Instruments that do not require ROX calibration.

Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96 and others.

Instruments requiring Low ROX calibration:

ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio® 5 System, QuantStudio® 6 Flex System, QuantStudio® 7 Flex System, ViiA 7 system. Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and more.

Instruments requiring High ROX calibration:

ABI Prism 7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, and others.

matters needing attention

1.Before using the reagents in this kit, please mix them gently by turning them up and down to avoid foaming as much as possible, and use them after brief centrifugation.

2.This product uses RNA as the template for one-step RT-PCR experiment, RNase contamination should be avoided during operation, it is recommended to operate RNA in a special area, use special instruments and consumables, the operator

with a mask and disposable gloves and often change the gloves, the experiment-related consumables should be processed with 0.1% DEPC (diethyl ether pyrocarbonate) aqueous solution for 12 hours at 37℃, and autoclaved for 30 minutes before use. The consumables should be treated with 0.1% DEPC (diethylpyrocarbonate) aqueous solution at 37℃ for 12 hours and autoclaved for 30 minutes.

3.Repeated freezing and thawing of each reagent in this kit should be avoided as much as possible; repeated freezing and thawing may degrade the product performance.

4.This kit must use specific primers, the choice of primers can be selected according to specific experiments, the good or bad primer design directly affects the results of RT-qPCR reaction, the design of primers need to consider the GC content, primer length, primer position, the secondary structure of the PCR product and other factors, it is recommended to use a professional primer design software for design.

5.This kit is recommended to use specific probes, and it is recommended to use professional design software for designing.

Usage

The following examples are conventional reaction systems and conditions, which should be improved and optimized according to the different templates, primer structures and target fragment sizes in actual operation. (Please prepare the reaction solution on ice.)

1. Dissolve RNA template, primers, 2× GoldStar Probe One Step Buffer, GoldStar Probe One Step EnzymeMix and RNase-Free Water and set aside on ice.

2. PCR reaction system:

reagents	25μl reaction system	final concentration
2×GoldStar Probe One Step Buffer	12.5μl	1×
Forward Primer, 10μM	0.5μl	0.2μM[1)]
Reverse Primer, 10μM	0.5μl	0.2μM[1)]
Probe, 10μM	0.5μl	0.2μM[2)]
GoldStar Probe One Step EnzymeMix	1.0μl	
RNA Template	Xμl	10pg-100ng[3)]
50 x Low ROX or High ROX (optional)[4)]	0.5μl	1×
RNase-Free Water	Up to 25μl	

Note: 1) Usually, better results can be obtained with a primer concentration of 0.2 μM, and 0.1-1.0 μM can be used as a reference for setting the range.

(2) The concentration of the probe used is related to the fluorescence quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance, please refer to the instrument manual or the specific requirements for the use of each fluorescent probe for the adjustment of the concentration in actual use.

(3) Usually the amount of RNA template is 10pg-100ng as a reference. Since the templates of different species contain different copy numbers of target genes, the templates can be diluted in gradient to determine the optimal amount of template to use.

(4) The excitation optical system varies from instrument to instrument, choose to add 50×Low ROX or 50×High ROX according to the instrument using fluorescence quantification.

3. Mix well, centrifuge briefly, and collect the solution at the bottom of the tube.

4.RT-PCR reaction conditions:

step	temperature	time	
reverse transcription	45° C	10min	
PCR pre denaturation	95° C	10min[1)]	
denaturation	95° C	15s	30-40 cycles
Annealing/Extension[2)]	60° C	45s	

Note: 1) The hot start enzyme used in this product must be activated under the condition of pre-denaturation 95℃, 5-10min. 2) It is recommended to use the two-step PCR reaction program, if you can not get good experimental results due to the use of primers with lower Tm value, etc., you can try to carry out the three-step PCR amplification, and the annealing temperature should be set in the range of 56℃-64℃ as a reference.