GoldStarProbeOneStepRT-qPCRKit

Project No. G665836

Storage conditions: -20° C.

Product Content:

Component	G665836 100rxns	G665836 100rxns	G665836 100rxns
2×GoldStarProbeOneStepBuffer	1.4ml	1.4ml	1.4ml
GoldStarProbeOneStepEnzymeMix	100 μl	100 μl	100 μl
50 x LowROX	-	50 μl	-
50 x HighROX	-	-	50 μl
RNase-FreeWater	1.5ml	1.5ml	1.5ml

Product Description:

This product is a specialized kit for one-step Real-TimeRTqPCR using the probe method (TaqMan, MolecularBeacon, etc.). When using this product for RealTimeRT-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 by using this product, more accurate quantification of target genes, good reproducibility and high confidence.ROX dye is used to correct the fluorescence signal error generated between wells of quantitative PCR instruments, and it is generally used in RealTimePCR amplifiers from ABI, Stratagene and other companies. The excitation optics of different instruments vary, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument.

Caveats:

1. Before using the reagents in this kit, please mix them gently by turning them up and down, avoid foaming as much as possible, and use them after brief centrifugation.
2. This product uses RNA as a template for one-step RT-PCR experiments, and RNase contamination should be avoided during operation.

It is recommended that RNA operations be carried out in a special area, with special instruments and consumables, and that operators wear masks and disposable gloves and change gloves frequently, and that consumables related to the experiments be treated with a 0.1% aqueous solution of DEPC (diethyl ether pyrocarbonate) for 12 hours at 37 ℃ and autoclaved for 30 minutes before use.

3. Avoid repeated freezing and thawing of the reagents in this kit, as repeated freezing and thawing may degrade the performance of the product.
4. This kit must use specific primers, primer selection can be selected according to specific experiments, primer design is good or bad 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 design.

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 OneStep 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	12.5 μl	1×
Forward Primer, 10 μM	0.5 μl	0.2 μM 1)
Reverse Primer, 10 μM	0.5 μl	0.2 μM 1)
Probe, 10 μM	0.5 μl	0.2 μM 2)
GoldStarProbeOne Step EnzymeMix	1.0 μl	/
RNATemplate	X μl	10 pg- 100 ng3)
50 x Low ROXor High ROX (optional)4)	0.5 μl	1×
RNase-Free Water	upto 25 μl	/

Attention:
(1) Usually, better results can be obtained with 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 material, please refer to the instrument manual or the specific requirements for the use of each fluorescent probe to adjust the concentration.

(3) Usually, the amount of RNA template is 10pg-100ng as a reference. Since the number of copies of target genes contained in the templates of different species is different, the templates can be subjected to gradient dilution to determine the optimal amount of template to be used.

(4) The excitation optical system varies from instrument to instrument, choose to add 50×LowROXor50× 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:

move	temp	timing	/
reverse transcription	45° C	10min	/
PCR pre-denaturation	95° C	10min	/
denaturation	95° C	15s	30-40 cycles
Annealing/Extension	60° C	45s	30-40 cycles

Attention:

(1) The hot-start enzyme used in this product shall be activated under the condition of pre-denaturation 95℃ and 5-10 min.

(2) It is recommended to use two-step PCR reaction program, if you can not get good results due to the use of primers with low Tm value, etc., you can try to do three-step PCR amplification, and the annealing temperature should be in the range of 56℃-64℃ as a setting reference.