

Note:

- 1) Generally, a primer concentration of 0.2 μ M can get better results, and 0.1-1.0 μ M can be used as a reference for the set 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 labeled substance. Please adjust the concentration according to the instructions of the instrument or the specific requirements for the use of each fluorescent probe.
- 3) Usually, the amount of RNA template is 10 pg-100 ng as a reference. Due to the different number of target gene copies contained in the template of different species, gradient dilution of the template can be carried out to determine the optimal template usage.
- 4) The excitation optical system of different instruments is different, and 50 \times Low ROX or 50 \times High ROX can be added according to the instrument using fluorescence quantification.

3. Mix well, centrifuge briefly, and collect the solution to the bottom of the tube.
4. Reaction conditions of RT-PCR:

Step	Temperature	Time
Reverse transcription	45°C	10 min
PCR predegeneration	95°C	10 min ¹⁾
Degeneration	95°C	15 s
Annealing/extension 2)	60°C	45 s

} 30-40 cycles

Note:

- 1) The thermal starting enzyme used in this product must be activated under the condition of predenaturation at 95°C for 5-10 min.
- 2) It is recommended to adopt the two-step PCR reaction procedure. If the primers with low T_m value are used and the experimental results are not good, the three-step PCR amplification can be attempted. Please set the annealing temperature in the range of 56°C to 64°C as the reference.

GoldStar Probe One Step RT-qPCR Kit

Cat. No. : CW2207S CW2632S CW2633S (100 rxns)

Storage Conditions: -20 °C away from light. For frequent use, store at 2-8 °C, avoid repeated freeze-thaw.

Components

Component	CW2207S 100 rxns	CW2632S 100 rxns	CW2633S 100 rxns
2 \times GoldStar Probe One Step Buffer	1.4 mL	1.4 mL	1.4 mL
GoldStar Probe One Step EnzymeMix	100 μ L	100 μ L	100 μ L
50 \times Low ROX	-	50 μ L	-
50 \times High ROX	-	-	50 μ L
RNase-Free Water	1.5 mL	1.5 mL	1.5 mL

Principle

This product is a special one-step Real-Time RT-qPCR kit using probe method (TaqMan, Molecular Beacon, etc.). When using this product for real-time RT-qPCR reaction, both reverse transcription and quantitative PCR are carried out in the same reaction system, and there is no need to add reagents or open the tube cover during the reaction process, which avoids contamination and improves the experimental efficiency. This product has high detection sensitivity, strong fluorescence signal and high signal-to-noise ratio, which is very suitable for the detection of RNA viruses and other trace RNA. It contains a special buffer system that makes reverse transcriptase identical to DNA polymerase when the maximum effect, improve the reaction efficiency. This product can be used to obtain a wider linear range, more accurate quantitative target cause, good repeatability, high credibility.

ROX dye is used to correct the fluorescence signal errors generated from hole to hole in quantitative PCR instrument. It is generally used in ABI, Stratagene and other companies' Real Time PCR amplifiers. The excitation optical system varies from instrument to instrument, so the concentration of ROX dye must be matched with the corresponding fluorescence quantitative PCR instrument.

Instruments without ROX correction: (CW2207)

Roche LightCycler 480, Roche LightCycler 96, Bio-rad iCycler iQ, iQ5, CFX96, etc.

Instruments requiring Low ROX correction: (CW2632)

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, etc.

Instruments requiring High ROX calibration: (CW2633)

ABI Prism7000/7300/770/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Cautions

1. Gently mix the reagents in this kit upside down before use, avoid foaming as far as possible, and use after a short centrifugation.
2. This product uses RNA as a template for one-step RT-PCR experiments. During the operation, RNase contamination should be avoided. Experimental consumables were treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous solution at 37°C for 12 hours and autoclaved for 30 minutes before use.
3. Reagents in this kit should avoid repeated freeze-thaw, which may degrade product performance.
4. This kit must use specific primers, and the selection of primers can be selected according to specific experiments. The design of primers directly affects the results of RT-qPCR.
5. Specific probe is recommended for this kit, and professional design software is recommended for design.

Procedure

The following examples are the conventional reaction system and reaction conditions, which should be improved and optimized according to the different template, primer structure and target segment size in actual operation. (Please configure the reaction liquid on the ice)

1. Dissolve the RNA template, primer, 2×GoldStar Probe OneStep Buffer, GoldStar Probe OneStep EnzymeMix and RNase-Free Water and place on ice for reserve.
2. PCR reaction system:

Reagent	25 µL Reaction system	Final conc.
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⁾
GoldStar Probe One Step EnzymeMix	1.0 µL	
RNA Template	X µL	10 pg -100 ng ³⁾
50×Low ROX or High ROX (optional) ⁴⁾	0.5 µL	1×
RNase-Free Water	up to 25 µL	