RT-qPCR reaction program (three-step method):

Steps	Temperature	Time	
Reverse Transcription	45 °C	10 min	
PCR Pre-Denaturation	95 °C	5 min	
Denaturation	95 °C	15 s	35-40 cycles
Annealing [1)]	56 °C-64 °C	30 s	
Extension	72 °C	30 s	
Melting Curve Analysis [2)]			
	95 °C	15 s	
	60 °C	1 min	
	95 °C	15 s	
	60 °C	15 s	

Note: 1) When the three-step PCR amplification is performed, the annealing temperature should be set in the range of 56 °C-64 °C.
2) The analysis of the melting curve should be set according to the recommended program of the fluorescence quantitative PCR instrument used. This program is set with the ABI 7500 fluorescence quantitative PCR instrument as the reference.

Version: 12/2023

UltraSYBR One Step RT-qPCR Kit

Cat. No. : CW0659S CW2623S CW2624S (100 rxns).

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

Components

Component	CW0659S 100 rxns	CW2623S 100 rxns	CW2624S 100 rxns
2×UltraSYBR One Step Buffer	1.4 mL	1.4 mL	1.4 mL
UltraSYBR One Step EnzymeMix	50 μL	50 μL	50 μL
50×Low ROX	-	50 μL	-
50×High ROX	-	-	50 μL
RNase-Free Water	1.5 mL	1.5 mL	1.5 mL

Introduction

This product is a one-step Real-Time RT-qPCR kit. The included SYBR Green I fluorescent dye binds to all double-stranded DNA, allowing the product to be used for the detection of a wide range of different target sequences without the need to synthesize specific labeled probes. Real Time RT-qPCR reactions, reverse transcription and quantitative PCR are performed in the same reaction system, and there is no need to add reagents or open the caps during the reaction, which avoids contamination and improves experimental efficiency. The new high-potency reverse transcriptase lacks RNase H activity, reducing RNA degradation in the reverse transcription reaction. The enzyme has a high reverse transcription efficiency and can perform a good reverse transcription reaction on a small amount of RNA template. It has high affinity with RNA, and can read through RNA templates with high GC content and complex secondary structure. The new high-efficiency hot-start enzyme blocks the activity of the enzyme at room temperature, thus effectively avoiding the non-specific amplification caused by the non-specific binding of primers and templates or primer dimers at room temperature, which greatly improves the accuracy of the real-time PCR reaction. The included buffer system maximizes the efficiency of both enzymes. This product has high sensitivity, high specificity, and a wide linear range, which makes the quantification of the target gene more accurate.

ROX dyes are used to correct the fluorescence signal errors generated between the wells of quantitative thermal cyclers and are generally used in Real Time PCR instruments from ABI, Stratagene, etc. The excitation optics vary from instrument to instrument, so the concentration of ROX must match the appropriate Real Time PCR instrument.

Instruments that do not require ROX calibration (CW0659):

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

Instruments requiring Low ROX calibration (CW2623):

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 (CW2624):

ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Precautions

1. Before using each reagent in this kit, gently invert it upside down to mix thoroughly, avoiding bubble formation as much as possible, and use it after brief centrifugation.
2. This product uses RNA as template for one-step RT-PCR experiments, therefore RNase contamination should be avoided during operation. It is recommended to perform RNA operations in a special area and use special instruments and consumables. Operators should wear masks and disposable gloves and change gloves frequently. Experiment-related consumables should be treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous solution at 37 °C for 12 hours, and autoclaved for 30 minutes before use.
3. UltraSYBR One Step RT-qPCR Buffer contains SYBR Green I fluorescent dye, and should be protected from strong light when storing this product or preparing PCR reactions.
4. The reagents in this kit should avoid repeated freeze-thaw, which may reduce the performance of the product. This product can be stored at -20 °C and protected from light for long-term storage. If frequent use is required in the short term, it can be stored at 2-8 °C.
5. This kit must use specific primers. Primers can be selected according to the specific experiment. The quality of primer design directly affects the results of RT-PCR reaction. When designing primers, factors to consider include GC content, primer length, primer location, and secondary structure of PCR products. It is recommended to use professional primer design software.
6. This product cannot be used for probe fluorescence quantitative PCR.

Protocol

1. Thaw the RNA template, primers, 2× UltraSYBR One Step Buffer, UltraSYBR One Step EnzymeMix and RNase-Free Water and set aside on ice.
2. PCR reaction system:

Reagent	25 μL	Final Concentration
2×UltraSYBR 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)]
UltraSYBR One Step EnzymeMix	0.5 μL	
RNA Template	X μL	10 pg - 100 ng
50×Low ROX or High ROX（optional）[2)]	0.5 μL	1×
RNase-Free Water	to 25 μL	

Note: 1) Usually the primer concentration of 0.2 μM can give good results, and the final concentration of 0.1-0.5 μM can be set as a reference for setting range. The primer concentration can be increased when the amplification efficiency is not high, and the primer concentration can be reduced in the case of non-specific reactions, so that the reaction system can be optimized.
2) The excitation optical systems may vary among different instruments, and 50×Low ROX or 50×High ROX should be added depending on the instrument used.

3. Vortex to mix, centrifuge briefly, and collect the solution to the bottom of the tube.
4. RT-qPCR reaction program (two-step method):
   This procedure is based on the ABI 7500 real-time PCR instrument as an example.

Steps	Temperature	Time	
Reverse Transcription	45 °C	10 min	
PCR Pre-Denaturation	95 °C	5 min	
Denaturation	95 °C	10 s	} 30-40 cycles
Annealing/Extension [1)]	60 °C	45 s	
Melting Curve Analysis [2)]			
	95 °C	15 s	
	60 °C	1 min	
	95 °C	15 s	
	60 °C	15 s	

Note: 1) It is recommended to use two-step PCR reaction procedure. If the specificity of the reaction should be increased, the annealing temperature can be increased, and the reference range of 60-64 °C can be used. If good experimental results are not obtained due to the use of primers with low Tm values, three-step PCR amplification can be attempted
2) The analysis of the melting curve should be set according to the recommended program of the fluorescence quantitative PCR instrument used. This program is set with the ABI 7500 fluorescence quantitative PCR instrument as the reference.