UltraSYBR One Step RT-qPCR Kit

Project number: U665751

Storage conditions: -20 ℃ to avoid light, such as the need for frequent use, can be stored in 2-8 ℃, try to avoid repeated freezing and thawing.

Product content

Component	U665751-100T
2×UltraSYBR One Step Buffer	1.4ml
UltraSYBR One Step EnzymeMix	50 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. The SYBR Green I fluorescent dye contained can bind to all double-stranded DNA, allowing this product to be used for the detection of many different target sequences without the need to synthesize specific labeling probes. Real Time RT-qPCR reaction using this product, reverse transcription and quantitative PCR are carried out in the same reaction system, there is no need to add reagents during the reaction, no need to open the cap of the tube, avoiding contamination while improving the efficiency of the experiment. The new high-efficiency reverse transcriptase RNase H is activity-deficient, which reduces the degradation of RNA in the reverse transcription reaction. The enzyme has high reverse transcription efficiency and can perform a good reverse transcription reaction on a small amount of RNA template. It has high affinity to RNA and can read through RNA templates with high GC content and complex secondary structure. New efficient hot start enzyme, the enzyme activity is closed at room temperature, thus effectively avoiding non-specific amplification caused by non-specific binding of primers and templates or primer dimerization at room temperature, which greatly improves the accuracy of fluorescence quantitative PCR reaction. The included buffer system maximizes the efficacy of both enzymes at the same time and improves efficiency. This product has high sensitivity, high specificity, wide linear range, and more accurate quantification of target genes.

ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used with 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, ViiA7 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 of pyrocarbonate) aqueous solution at 37℃ for 12 hours and autoclaved for 30 minutes before use. Sterilize for 30 minutes before use.
3. UltraSYBR One Step RT-qPCR Buffer contains SYBR Green I fluorescent dye. Avoid bright light when storing this product or preparing PCR reaction solutions.
4. Repeated freezing and thawing of each reagent in this kit should be avoided; repeated freezing and thawing may degrade the product performance. This product can be stored for a long time at -20℃, protected from light. If frequent use is required in the short term, it can be stored at 2-8℃.
5. 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-PCR 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.
6. This product cannot be used for fluorescent quantitative PCR by the probe method.

Usage

1. Dissolve 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:

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

Note: 1) Usually, the primer concentration of 0.2μM can get better results, and the final concentration of 0.1-0.5μM can be used as a reference for setting the range. If the amplification efficiency is not high, the concentration of primer can be increased; when non-specific reaction occurs, the concentration of primer can be decreased, thus optimizing the reaction system.
(2) 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. Vortex and shake to mix, centrifuge briefly, and collect the solution at the

bottom of the tube.

4. RT-qPCR reaction conditions (fluorescence quantitative PCR is a two-step method), this program is based on the ABI 7500 fluorescence quantitative PCR instrument as an example.

Steps	Temperature	time	
reverse transcription	45° C	10min	
PCR pre-denaturation	95° C	5min	
denaturation	95° C	10s	30-40 cycles
Annealing/Extension[1)]	60° C	45s	
Analysis of fusion curve[2)]			
	95° C	15s	
	60° C	1min	
	95° C	15s	
	60° C	15s	

Note: 1) It is recommended to use two-step PCR reaction program, if you improve the reaction specificity, you can increase the annealing temperature to 60-64 ℃ as a reference for the setting range; if you do not get good experimental results due to the use of primers with lower Tm values, etc., you can try to carry out three-step PCR amplification.

(2) For melting curve analysis, please set up the program recommended by the fluorescence quantitative PCR instrument used, and this program is set up with the ABI 7500 fluorescence quantitative PCR instrument as a reference.

RT-qPCR reaction conditions (fluorescence quantitative PCR was a three-step method):

Step	Temperature	time	
reverse transcription	45° C	10min	
PCR pre-denaturation	95° C	5min	
denaturation	95° C	15s	35-40 cycles
Annealing[1)]	56° C-64° C	30s	
Extend	72° C	30s	
Analysis of fusion curve[2)]			
	95° C	15s	
	60° C	1min	
	95° C	15s	
	60° C	15s	

Note: 1) For three-step PCR amplification, please use the range of 56℃-64℃ as the setting reference for the annealing temperature.

(2) For melting curve analysis, please set up the program recommended by the fluorescence quantitative PCR instrument you are using, this program is ABI 750 fluorescent quantitative PCR instrument as a reference setting.