UltraSYBR One Step RT-qPCR Kit

UltraSYBR One-Step Fluorescence PCR Kit

Item No. U665567 (100 rxns)
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

individual parts making up a compound	U665567 100 rxns
2×UltraSYBR One Step Buffer	1.4 mL
UltraSYBR One Step EnzymeMix	50 μL
50 x Low ROX	/
50 x High ROX	/
RNase-Free Water	1.5 mL

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 good reverse transcription reaction on a small amount of RNA template. High affinity to RNA, can read through RNA templates with high GC content and complex secondary structure. The new high efficient hot start enzyme, the activity of the enzyme 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 the 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 quantitative PCR instruments, and it is generally used in Real Time PCR amplifiers of ABI, Stratagene, etc. 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, etc.

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 others.

Instruments requiring High ROX calibration: ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Caveat

1. Before using the reagents in this kit, please mix them gently by turning them upside down to avoid foaming as much as possible, and use them after centrifugation for a short period of time.
2. This product uses RNA as the template for one-step RT-PCR experiment, RNase contamination should be avoided during the operation, it is recommended to operate RNA in a special area, use special instruments and consumables, the operator should wear a mask and disposable gloves and change the gloves frequently, and the consumables related to the experiment should be processed with 0.1% DEPC (diethyl ether of pyrocarbonate) aqueous solution at 37℃ for 12 hours and be autoclaved for 12 minutes before use. Autoclave for 30 minutes before use.
3. UltraSYBR One Step RT-qPCR Buffer contains SYBR Green I Fluorescent Dye, so avoid bright light when storing this product or preparing PCR reaction solution.
4. Avoid repeated freezing and thawing of the reagents in this kit, which may degrade the performance of the product. The product can be stored at -20℃ for a long time and protected from light. If the product needs to be used frequently in a short period of time, it can be stored at 2-8℃.
5. 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-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 professional primer design software for design.
6. This product cannot be used in the probe method of fluorescence quantitative PCR.

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	25 μL reaction system	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 x Low ROX or High ROX (optional)[2)]	0.5 μL	1×
RNase-Free Water	up to 25 μL	/

Attention:
(1) Usually, a primer concentration of 0.2 μM can give better results, and a final concentration of 0.1-0.5 μM can be used as a reference for setting the range. If the amplification efficiency is not high, the concentration of primer can be increased; if non-specific reaction occurs, the concentration of primer can be decreased to optimize 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 to ABI 7500 fluorescence quantitative PCR instrument as an example.

move	temp	timing	/
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	30-40 cycles
Melting curve analysis[2)]	/	/	/
/	95° C	15 s	/
/	60° C	1 min	/
/	95° C	15 s	/
/	60° C	15 s	/

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

move	temp	timing	/
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	35-40 cycles
reach	72° C	30 s	35-40 cycles
Melting curve analysis[2)]	/	/	/
/	95° C	15 s	/
/	60° C	1 min	/
/	95° C	15 s	/
/	60° C	15 s	/

Attention:

(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 used, and this program is set up with the ABI 7500 fluorescence quantitative PCR instrument as a reference.