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One Step RT-qPCR SYBR Kit

Product Number: PCK59

Shipping and Storage

-20°C to avoid light, if need frequent use, can be stored in 2-8°C, try to avoid repeated freeze-thaw..

Components

Component	PCK59 100rxns	PCK59L 100rxns	PCK59H 100rxns
2×One Step RT-qPCR SYBR Buffer	1.4mL	1.4mL	1.4mL
One Step RT-qPCR SYBR EnzymeMix	50μL	50μL	50μL
50×Low ROX	-	50μL	-
50×High ROX	-	-	50μL
RNase-Free Water	1.5mL	1.5mL	1.5mL

Description

This product is a one-step Real-Time RT-qPCR special kit. The SYBR Green I fluorescent dye can be combined with all double-stranded DNA, enabling the product to be used for the detection of a variety of different target sequences without the need for the synthesis of an hetero-labeled probe. This product is used for Real Time RT-qPCR reaction. Reverse transcription and quantitative PCR are carried out in the same reaction system. There is no need to add reagents or open the tube cover during the reaction process, which avoids contamination and improves the experimental efficiency. A new, highly effective reverse transcriptase, RNase H, was absent, reducing the degradation of RNA in the reverse transcriptase reaction. This enzyme has high reverse transcriptional efficiency and can perform a good reverse transcriptional response to a small amount of RNA template. High affinity with RNA, able to read through the RNA template with high GC content and complex secondary structure. The activity of the enzyme is blocked at room temperature, thus effectively avoiding the nonspecific amplification caused by the nonspecific binding of primer and template or primer dimer at room temperature, which greatly improves the accuracy of fluorescence quantitative PCR reaction. The contained buffer system enables both enzymes to function simultaneously Maximum effect, improve efficiency. This product has high sensitivity, high specificity, wide linear range and more accurate quantitative target genes.

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

Instruments requiring Low ROX correction: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:ABI Prism7000/7300/770/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Notes

1. Please mix all reagents in this kit upside down gently 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 experiment. During operation, RNase contamination should be avoided. It is recommended that RNA operation should be carried out in special areas, special instruments and consumables should be used, and operators should wear masks and primary gloves and change gloves frequently. 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. The One Step RT-qPCR SYBR Buffer contains SYBR Green I fluorescent dye, and strong light should be avoided when storing this product or preparing PCR reaction solution.
4. The reagents in this kit should avoid repeated freeze-thaw, which may degrade product performance. This product can be stored for a long time at -20°C, away from light. If you need to use frequently in the short term, it can be stored at 2-8°C.
5. Specific primers must be used in this kit, and the selection of primers can be selected according to specific experiments. The design of primers directly affects the results of RT-PCR reaction. When designing primers, GC content, primer length, primer location, secondary structure of PCR products and other factors should be considered.
6. This product cannot be used for probe fluorescence quantitative PCR.

Protocol

1. The RNA template, primer, 2×One Step RT-qPCR SYBR Buffer, One Step RT-qPCR SYBR EnzymeMix and RNase-Free Water were dissolved and placed on ice for reserve.
2. PCR reaction system:

Reagent	25μL reaction system	Final Concentration
2×One Step RT-qPCR SYBR Buffer	12.5μL	1×
Forward Primer，10μM	0.5μL	0.2 μM[1)]
Reverse Primer，10μM	0.5μL	0.2 μM[1)]
One Step RT-qPCR SYBR EnzymeMix	0.5μL	
RNA Template	XμL	10pg – 100ng
50×Low ROX or High ROX (optional)[2)]	0.5μL	1×
RNase-Free Water	up to 25μL	

Note: 1) Generally, a primer concentration of 0.2μM can obtain better results, and the final concentration of 0.1-0.5μM can be used as a reference for the set range. When the amplification efficiency is not high, the concentration of primers can be increased. When non-specific reaction occurs, the concentration of primers can be reduced to optimize the reaction system.

2)The excitation optical system of different instruments is different, and 50×Low ROX or 50×High ROX can be added according to the instrument using fluorescence quantification.

3. The solution is collected to the bottom of the tube by vortex mixing and temporary centrifugation.
4. Reaction conditions of RT-qPCR (fluorescence quantitative PCR is a two-step method). This procedure takes ABI 7500 fluorescence quantitative PCR instrument as an example.

Step	Temperature	Time	
reverse transcription	45°C	10min	
PCR pre denaturation	95°C	5min	
degeneration	95°C	10s	30-40cycles
annealing/extension[1)]	60°C	45s	
melting curve analysis[2)]			
	95°C	15s	
	60°C	1min	
	95°C	15s	
	60°C	15s	

Note: 1) It is recommended to use the two-step PCR reaction procedure. If the reaction specificity is improved, the annealing temperature can be increased, and 60-64°C is used as the reference range. If the primers with low Tm value are not good experimental results, the three-step PCR amplification can be tried.

2)The melting curve analysis should be set according to the procedure recommended by the fluorescence quantitative PCR instrument used. This procedure is set according to the ABI 7500 fluorescence quantitative PCR instrument.

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

Step	Temperature	Time	
reverse transcription	45°C	10min	
PCR pre denaturation	95°C	5min	
degeneration	95°C	15s	30-40cycles
Annealing[1)]	56°C-64°C	30s	
extension	72°C	30s	
melting curve analysis[2)]			
	95°C	15s	
	60°C	1min	
	95°C	15s	
	60°C	15s	

Note: 1) For three-step PCR amplification, the annealing temperature should be set in the range of 56°C-64°C.

2) The melting curve analysis shall be set according to the procedure recommended by the fluorescence quantitative PCR instrument used, which is set according to the fluorescence quantitative PCR instrument ABI7500.