ClearPeak One Step qRT-PCR SYBR Kit

100 rxn

Cat No: 1SQ100

Shipping : Ship with blue ice.
Storage : Store at −20°C, protected from light.
For frequent use, the product may be stored at 2–8 °C. Avoid repeated freeze–thaw cycles.

General Information

ClearPeak One Step qRT-PCR SYBR Kit is a one-step Real-Time RT-qPCR kit designed for rapid and sensitive detection of RNA targets. It utilizes SYBR Green I dye, which binds to double-stranded DNA, allowing detection of a wide range of target sequences without the need for sequence-specific probes.

Reverse transcription and quantitative PCR are performed consecutively within a single reaction mixture, eliminating the need for tube opening or additional reagent handling. This closed-tube format minimizes contamination risk and enhances workflow efficiency.

ClearPeak One Step qRT-PCR SYBR Kit includes an advanced reverse transcriptase lacking RNase H activity, thereby preserving RNA integrity during cDNA synthesis. The enzyme exhibits high efficiency even with low RNA input and demonstrates strong performance with templates containing high GC content or complex secondary structures.

To improve specificity, the reverse transcriptase remains inactive at lower temperatures and is activated during the initial stages of thermal cycling. This feature helps reduce non-specific amplification caused by primer–dimer formation or non-specific primer binding, leading to improved accuracy in quantitative analysis.

The optimized buffer system enables both reverse transcription and PCR amplification to occur efficiently within the same reaction, maximizing overall performance. The kit offers high sensitivity, excellent specificity, and a broad dynamic range for precise quantification of target genes.

ROX reference dye is included to normalize fluorescence signal variations between wells. As different Real-Time PCR instruments require different ROX concentrations, users should select the appropriate setting based on their instrument specifications.

No ROX correction required: Roche LightCycler® 480, LightCycler® 96, Bio-Rad iCycler iQ, iQ5, CFX96

Low ROX required: ABI Prism® 7500/7500 Fast, QuantStudio® 3, 5, 6 Flex, 7 Flex, ViiA™ 7, Stratagene Mx3000/Mx3005P, Rotor-Gene® 3000

High ROX required: ABI Prism® 7000, 7300, 7700, 7900, ABI StepOne™ / StepOnePlus™, Eppendorf systems.

Instructions for using ROX:

ClearPeak 100x ROX Reference Dye should be added directly to the *ClearPeak* 2x One Step qRT-PCR SYBR Buffer according to the ROX requirement of your real-time PCR instrument.

For 1 mL of *ClearPeak* 2x One Step qRT-PCR SYBR Buffer, add:

High ROX instruments: add 20 µL of *ClearPeak* 100X ROX Reference Dye
Low ROX instruments: add 2 µL of *ClearPeak* 100X ROX Reference Dye
No ROX instruments: do not add ROX

After adding ROX, mix gently but thoroughly before use. Avoid repeated freeze–thaw cycles of the ROX-premixed buffer.

For further information;
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Protocol

1. Thaw the RNA template, primers, *ClearPeak* 2x One Step qRT-PCR SYBR Buffer (ROX added according to the above protocol), *ClearPeak* One Step qRT-PCR SYBR Enzyme Mix, and RNase-free water, and keep all components on ice until use.

Important Notes:

- Please gently mix the tube upside down before use and avoid foaming as much as possible. Avoid repeated freeze-thaw, as repeated freeze-thaw may cause a decrease in product performance.
- Due to the presence of fluorescent dye SYBR Green I in this product, it is necessary to store it away from light. to avoid strong light exposure when preparing the reaction.

2. Prepare the following reaction mixture

Component	Amount
ClearPeak 2x One Step qRT-PCR SYBR Buffer	10 µl
ClearPeak One Step qRT-PCR SYBR Enzyme Mix	1 µl
Forward Primer (10µM)	0.4 µl
Reverse Primer (10µM)	0.4 µl
RNA Template	<100ng
ddH_2O	up to 20 µl
Total	20 µl

Important Notes:

- Typically, the final concentration of the primer is 0.2 µM can achieve successful results, where a final concentration of 0.1-1.0µM primer may serve as a reference for setting the range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction.

3. Use the following reaction conditions to run the experiment. It is recommended to use a two-step PCR reaction program. If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification can be attempted.

Two Step

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	15 min	
Initial Denaturation	94°C	2 min	
Denaturation	94°C	15 sec	35-40
Annealing/Extension	60°C	15-30 sec	

Three Step

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	15 min	
Initial Denaturation	94°C	2 min	
Denaturation	94°C	15 sec	
Annealing	55-65°C	15 sec	35-40
Extension	72°C	30 sec	

Important Notes:

- In practical operation, corresponding improvements and optimizations should be made to PCR Setup and PCR Reaction Conditions based on different templates, primer structures, and target fragment sizes.