One-step Universal Green RT-qPCR Kit

Product description

Quantitative PCR (qPCR), also known as Real-time PCR, is a highly versatile and precise technique for analyzing gene expression. According to different principles, qPCR can be divided into two categories: dye-based methods and probe-based methods. Among the two methods, dye-based methods are more universal, convenient, and cost-effective. Dye-based qPCR indirectly measures DNA amplification during each PCR cycle by monitoring the fluorescence emitted by dyes that bind specifically to double-stranded DNA (dsDNA). When the fluorescence signal detected at a certain time point significantly exceeds the background, the Ct value (also known as Cq value) can be determined. The obtained Ct values can be used to evaluate the relative abundance of the target gene or to calculate absolute quantities based on an appropriate standard curve.

One step RT-qPCR provides a convenient and efficient method for the detection and quantification of RNA. In a single tube, RNA is first converted into cDNA by reverse transcriptase, and then amplified using DNA polymerase for qPCR quantitative analysis.

Our product utilizes the dsDNA-binding dye SYBR Green I to monitor DNA amplification after each PCR cycle, enabling dye-based real-time quantitative analysis of target RNA sequences. The SYBR Green RT-qPCR Enzyme Mix in this kit contains antibody- -mediated hot-start Taq DNA polymerase, a novel reverse transcriptase with high thermal stability and Rnase inhibitor. The recommended reverse transcription temperature is 55°C, but it can be adjusted between 50°C -55°C. The 2× SYBR Green RT-qPCR Reaction Buffer contains dNTPs, SYBR Green I, and all necessary buffer components. Additionally, the premix includes a unique inert reference dye compatible with various qPCR instruments, including those requiring high or low ROX reference signals. Furthermore, the premix employs a thermolabile UDG+dUTP anti-contamination system, ensuring the accuracy of qPCR amplification results and reducing false-positive results caused by aerosol contamination. The kit also contains a non-fluorescent visible tracking dye, facilitating the visualization of reaction setup without interfering with real-time detection.

However, dye-based qPCR has certain limitations. SYBR Green I can intercalate into any double-stranded DNA, including primer dimers or other non-specific products, causing these non-specific products to emit fluorescence. Therefore, to confirm the specificity of the amplified products, it is necessary to perform melting curve analysis after amplification. In

melting curve analysis, an ideal experimental result is indicated by a single sharp peak near the primer annealing temperature.

Composition and storage conditions

Components \ Size	100 rxns	200 rxns	500 rxns	1000 rxns	Storage
SYBR green RT-qPCR Enzyme Mix	100 μL	200 μL	500 μL	1 mL	-20°C
2×SYBR Green RT-qPCR Reaction Buffer	1 mL	2×1 mL	5×1 mL	10×1 mL	-20°C
Nuclease-free Water	1 mL	2×1 mL	5×1 mL	10×1 mL	-20°C

Shipping: Dry Ice **Shelf life:** 12 months

Experimental operation

1. Reaction Setup

a) Thaw 2×SYBR Green RT-qPCR Reaction Buffer, primers and Nuclease-free Water at room temperature, thaw the RNA template on ice, then mix thoroughly and centrifuge, and place on ice for later use.

b) Set up the reaction system according to the following table:

Reagent	20 μL Reaction	Final Concentration
2×SYBR Green RT-qPCR Reaction Buffer	10 μL	1×
SYBR green RT-qPCR Enzyme Mix	1 μL	1×
Forward primer (10 μM)	0.5 μL	0.25 μM
Reverse primer (10 μM)	0.5 μL	0.25 μM
Template RNA	variable	< 1 μg (total RNA)
Nuclease-free Water	to 20 μL	

***Note**

a. It is recommended to use pipette tips with filters. Avoid cross-contamination and aerosol contamination.

b. To obtain the best results, it is recommended that each sample be repeated three times.

c. Usually, the final concentration of the primer is 0.25 μmol/L. It can also be adjusted within the range of 0.1-1.0 μmol/L according to the situation. Note to dissolve the primer using Nuclease-free Water.

d. When there are a large number of samples to be tested, a mixture of the same reagents as required can be prepared first based on the quantity of samples, usually by 10% more. Then, it is aliquot into each reaction well. Finally, different reagents are added to each reaction well. This can make the volume of reagents taken more accurate and uniform, with smaller errors and reduced reagent loss.

c) Invert, or gently pipette or gently vortex to mix the solution thoroughly, and briefly centrifuge to collect the liquid to

the bottom of the tube.

d) Place the reaction tube or reaction plate on the qPCR instrument and proceed to the next step of reaction setup.

2. qPCR Cycling Conditions

Set up the reaction program according to the table below. Use the SYBR or SYBR/FAM mode of the qPCR instrument to ensure that the fluorescence signal is collected at the end of the extension step.

Procedure	Temperature	Time	Cycles
Reverse Transcription	55°C	10 min	1
Initial Denaturation	95°C	1 min	1
Denaturation	95°C	10 s	40-45
Extension	60°C	30-60 s	1
Melt Curve	60-95°C	various	1

***Note:** The actual extension time can be adjusted according to the reading time of the qPCR instrument.

Notes

1. **Primer Design Guidelines:** The primers for qPCR experiment should have good amplification effects and few non-specific products. You can make reference to the following design guidelines:

1) **Target sequence length:** 80-200 bp is an ideal length. It can be extended to 300 bp according to actual requirements.

2) **Primer length:** 17-30 bp.

3) **GC content**: 40-60% (ideal 45-55%)

4) **Tm:** The Tm values of the forward primers and reverse primers should not have significant differences. The Tm values can be calculated by software.

5) **Primer sequence:** The distribution of A, T, C, and G should be relatively uniform, avoiding regions with particularly high GC or AT content (especially at the 3 'end), and avoiding polypyrimidine (T/C continuous structure) and polypurine (A/G continuous structure).

6) **3' terminal sequence**: The GC and AT contents at the 3' terminal of the primer should not be too high. We suggest choosing sequences with G or C at the 3' end (avoiding those with T at the 3' end). Complementary sequences of more than three bases cannot exist in primers or between primer pairs (respectively causing hairpin structures or primer dimers). Primer pairs should not have complementary sequences of more than two bases at each 3' end to avoid primer dimers.

7) **Specificity:** The specificity of the primers is confirmed through software. When designing primers, input sufficient

sequences around the region of interest. Employ search criteria that allow cross-reference to relevant sequence databases (to avoid potential amplification off-target).

2. Reaction conditions

1) For 96-well plates, we recommend using a reaction volume of 20 μL. The 384-well plate is recommended to have a reaction volume of 10 μL.

2) When setting up the Cycling program, it is necessary to ensure that a signal detection program is included at the end of the extension step and a melting curve analysis is conducted at the end to determine the specificity of the product.

3) Amplifying 40 cycles is sufficient for most experiments, but for target genes with extremely low copy in the sample, 45 cycles can be used.

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