UniPeak U+ One Step RT-qPCR SYBR Green Kit

Q226

Version 24.2

Product Description

UniPeak U+ One Step RT-qPCR SYBR Green Kit is a dye-based RT-qPCR reagent specifically designed for quantitative PCR detection using RNA as the template. The dye-based fluorescence quantitative detection presents two major challenges: the difficulty of detecting low-concentration samples and the issue of non-specific amplification. This product addresses these challenges with a next-generation antibody-modified Taq DNA Polymerase and a one-step, temperature-activated Reverse Transcriptase, combined with an optimized buffer system for RT-qPCR, offering enhanced sensitivity and specificity. To further ensure accurate quantification, a dUTP/UDG anti-contamination system is included, eliminating aerosol contamination at room temperature. Additionally, the 5 × gDNA Wiper effectively removes any residual genomic DNA contamination from RNA templates. The 2 × One Step SYBR Green Master Mix includes tracking dye and universal ROX Reference Dye, making it compatible with all types of qPCR instruments, requiring only the addition of primers and templates for amplification.

Components

	Components	Q226-01 250 rxns (20 μl/rxn)
■	5 × gDNA Wiper Mix	1 ml
■	2 × One Step SYBR Green Master Mix*	2 × 1.25 ml

* Includes Reverse Transcriptase, Taq DNA Polymerase, RNase Inhibitor, Heat-labile UDG, dUTP, dNTPs, Mg^{2+}, SYBR Green I, Specific ROX Reference Dye, blue dye, etc.

Storage

Store at -30 ~ -15°C and protect from light. Ship at ≤0°C.

Applications

It is applicable for animals, plants, microorganisms (viruses, etc.) various RNA nucleic acid testing.

Notes

1. 2 × One Step SYBR Green Master Mix contains SYBR Green I and ROX dye, please mix thoroughly and centrifuge before use.
2. 2 × One Step SYBR Green Master Mix should avoid strong light exposure during use, and note to protect from light for storage.
3. For the preparation of the reaction solution, please use RNase-free pipette tips, RNase-free centrifuge tubes, etc., to minimize contamination.

Experiment Process (Using ABI QuantStudio 3 as the Test Model)

1. Genomic DNA Removal from RNA Template (Optional)

Prepare the following reaction mixture in an RNase-free microcentrifuge tube:

Components	Volume	
RNase-free ddH_2O	to 20 μl	
5 × gDNA Wiper Mix	4 μl	■
Template RNA	Total RNA: 1 pg - 1 μg	

After gently mixing by pipetting up and down or briefly vortexing, centrifuge the tube, and incubate at 42°C for 2 min.

▲ Do not premix this component with primers in advance.

▲ This step is optional. If the removal of genomic DNA is not required, it can be omitted.

2. Preparation of RT-qPCR Reaction Mixture

Prepare the following reaction mixture in an RNase-free microcentrifuge tube:

Components	Volume
2 × One Step SYBR Green Master Mix	10 μl
Primer Forward (10 μM)	0.4 μl
Primer Reverse (10 μM)	0.4 μl
Template RNA	Total RNA: 1 pg - 1 μg
RNase-free ddH_2O	to 20 μl

The amounts of each component in the reaction mixture can be adjusted based on the following principles:

▲ For RNA templates treated with gDNA wiper (Step 1), add 1 - 9.2 μl of the first-step reaction mixture, adjusting according to the template concentration to ensure a final input ranging from 1 pg to 1 μg. The recommended optimal addition volume is 2 - 5 μl.

▲ In general, a final primer concentration of 0.2 μM in the reaction mixture yields optimal amplification results. If the reaction performance is suboptimal, the primer concentration can be adjusted within the range of 0.1 to 1.0 μM.

▲ qPCR is highly sensitive, and the accuracy of the template amount added to the reaction mixture significantly impacts the final quantification results. Therefore, it is recommended to dilute the template (e.g., to 2 - 5 μl per sample) before digestion or to add the diluted template directly into the amplification reaction mixture. This practice can effectively improve the reproducibility of the experiment.

3. Reaction Program

Standard Program

Steps	Temperature	Time	Cycles
Reverse Transcription	50 ~ 55°C	5 min*	
Initial Denaturation	95°C	30 sec	
Denaturation	95°C	10 sec	40
Annealing Extension	60°C	30 sec	
Melt Curve		Instrument Default	

* The reverse transcription duration can be extended to 15 min to enhance cDNA yield.

Fast program

In the "Experiment Properties" interface, select the "Fast" mode under "Run mode".

Steps	Temperature	Time	Cycles
Reverse Transcription	50 ~ 55°C	3 min*	
Initial Denaturation	95°C	30 sec	
Denaturation	95°C	5 sec	40
Annealing Extension	60°C	20 sec	
Melt Curve		Instrument Default	

* The reaction times and temperature ramp rates for each phase in the fast protocol can be adjusted based on the specific real-time PCR instrument used and individual requirements.

4. After the reaction, confirm the amplification curve and melting curve of the Real-Time PCR, and proceed with the creation of the standard curve.