One-step Universal Probe 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 the principle, qPCR can be divided into dye-based and probe-based methods. Commonly used probes include TaqMan probes, molecular beacons, dual-hybridization probes, MGB probes, etc. Among these, TaqMan probes are the most classical and widely used. TaqMan probes specifically hybridize to the target sequence and are labeled with a fluorescent reporter dye at the 5' end and a quencher dye at the 3' end. When the probe is intact, the fluorescence of the reporter dye is quenched due to Fluorescence Resonance Energy Transfer (FRET). During primer-mediated extension, the 5'→3' exonuclease activity of Taq polymerase cleaves the probe bound to the target sequence, separating the fluorescent reporter dye from the quencher dye. Once separated by more than 10 nm, the reporter dye emits fluorescence upon excitation. With each PCR cycle, more fluorescent reporter dyes are released, and the fluorescence intensity is proportional to the amount of newly synthesized target fragments, enabling detection. Common fluorescent reporter dyes at the 5' end include FAM, HEX, TET, JOE, NED, Cy3, TAMRA, Cy5, Cal Fluor Gold540, Cal Fluor Orange560, etc. Common quencher dyes at the 3' end include BHQ1, BHQ2, BHQ3, TAMRA, Dabcyl, NFQ-MGB, etc. When designing probes, select appropriate dyes according to the qPCR instrument, target gene, and experimental type.

One-step Universal Probe RT-qPCR Kit is a one-step real-time fluorescent quantitative PCR premix based on the probe method, designed for detecting target RNA. Its working principle is as follows: first, reverse transcriptase converts RNA into cDNA; subsequently, DNA polymerase amplifies the cDNA. The entire process is completed in a single tube.

This product employs a thermolabile UDG+dUTP anti-contamination system, ensuring accurate qPCR amplification results and reducing false positives caused by aerosol contamination. 2× Probe RT-qPCR Reaction Buffer contains specially formulated ROX reference dye, suitable for all qPCR instrument platforms without adjusting ROX concentration, ensuring consistent and reliable results. It also contains a non-fluorescent visible tracking dye for easy observation during reaction setup, which does not interfere with real-time detection.

This product is suitable for pathogen detection, RNA virus detection, gene expression analysis, and other applications.

Composition and storage conditions

Components \ Size	K1551-100 rxns	K1551-200 rxns	K1551-500 rxns	K1551-1000 rxns	Storage
Probe RT-qPCR Enzyme Mix	100 µL	200 µL	500 µL	1 mL	-20°C
2×Probe 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 Protocol

1. Reaction Setup

a) Thaw the 2×Probe RT-qPCR Reaction Buffer, primers and Nuclease-free Water at room temperature, the probes and RNA should be thawed on ice. After thorough mixing and centrifugation, place them on ice for later use.

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

Singleplex qPCR:

Reagent	20 µL Reaction	Final Concentration
2×Probe RT-qPCR Reaction Buffer	10 µL	1×
Probe RT-qPCR Enzyme Mix	1 µL	1×
Forward Primer (10 µM)	0.6 µL	0.3 µM
Reverse Primer (10 µM)	0.6 µL	0.3 µM
Probe (10 µM)	0.4 µL	0.2 µM
Template RNA	variable	< 1 µg (total RNA)
Nuclease-free Water	To 20 µL	

***Note**

a. Usually, a final primer concentration of 0.3 µM in the reaction system can achieve a good amplification effect. If the reaction performance is poor, the primer concentration can be adjusted within the final concentration range of 0.1-1 µM. The final concentration adjustment range of the probe is 0.05-0.5 µM. Note to dissolve the primers and probes using Nuclease-free Water.

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

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

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

Multiplex qPCR:

Reagent	20 μL Reaction	Final Concentration
2×Probe RT-qPCR Reaction Buffer	10 μL	1×
Probe RT-qPCR Enzyme Mix	1 μL	1×
Forward Primer (10 μM)	X μL	0.1 - 1 μM
Reverse Primer (10 μM)	X μL	0.1 - 1 μM
Probe (10 μM)	X μL	0.05 - 0.5 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. Up to quadruplex qPCR tests can be conducted at most.

c. When conducting multiplex qPCR, it is important to control the primer concentration. Especially for the target gene with high abundance, it is necessary to pay attention to reducing the primer concentration.

d. When designing multiplex qPCR probes, it is first necessary to determine the fluorescent groups according to different models of qPCR instruments, and to pay attention to reducing the mutual interference of fluorescence emission spectra among different probes.

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

f. 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 adding 10% overage. Then, it is aliquot into each reaction well. Finally, different reagents are added to each reaction well. This can make the volume of taken reagents more accurate and uniform, with smaller errors and reduced reagent loss.

c) Invert, or gently pipette or gently vortex to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube.

2. qPCR Cycling Conditions

When setting up the qPCR instrument program, pay attention to the consistency of the selected fluorescence channel with the fluorescent group and quencher group of the probe, and ensure that the fluorescence signal is collected at the end of the extension step. Some qPCR instruments have Fast programs. If needed, you can directly select the Fast program first and then modify the program (such as ABI 7900HT Fast, QuantStudio series, StepOne, StepOnePlus, ViiA™ 7, 7500 Fast, etc.).

Standard Program:

Cycle Step	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	

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

Fast Program:

Cycle Step	Temperature	Time	Cycles
Reverse Transcription	55°C	10 min	1
Initial Denaturation	95°C	1 min	1
Denaturation	95°C	3 s	40-45
Extension	60°C	20-34 s	

***Note:** Please set up the program according to the fast mode of the actual used qPCR instrument. The minimum extension time shall be no less than 20 seconds.

Notes

1. **Primer Design Guidelines:** It is recommended to make reference to the following design guidelines:

1) **Primer length**: 17-25 bp is optimal. Too short primers can easily lead to a decrease in amplification efficiency. If the primer is too long, the probability of the advanced structure formation of the primer will increase. Both will interfere with the accuracy of the quantitative results.

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

3) **Tm**: The Tm values of the forward primer and the reverse primer should not have a significant difference, and it is best that the difference does not exceed 2℃. The optimal Tm value is around 60℃.

4) **Primer sequence:** The distribution of A, T, C, and G should be relatively uniform. Areas with particularly high GC or AT content should be avoided.

5) **3' sequence:** Avoid more than 2 G or C nucleotides in the last 5 nucleotides at the 3' end to reduce the risk of non-specific amplification.

2. **TaqMan Probe Design Guidelines:**

1) **Probe sequence:** It is recommended to be closed to the forward or reverse primers, but there should be no overlapping regions with them.

2) **Probe length:** Generally 20-40 bp.

3) **GC content:** 30-60%

4) **Characteristics of the probe sequence**: Consecutive identical bases should be avoided, especially GGGG or more consecutive G. If the sequence contains polymorphic sites, they should be located in the middle of the probe sequence.

5) **Probe 5' end:** Base G should be avoided. Because the 5 'end is connected to the fluorescent group, the G base will quench the fluorescence signal of the fluorescent group. Even if only one G base remains connected to the reporter group after hydrolysis, this quenching effect still exists.

6) **Probe annealing temperature:** It should be 65 to 70 ℃. It is recommended to be 5-10 ℃ higher than the Tm value of the primer.

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

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com