Probe RT-qPCR Kit Series

User's Instruction

Description

Probe RT-qPCR Kit Series are specifically developed based on the fluorescent quantitative PCR technique by the probe method, and they have the following characteristics:

- Ready to use, users only need to provide sample RNA template.
- The primers and probes have been optimized with high sensitivity.
- Positive controls are provided to distinguish false negative samples.
- Highly specific. Primers are designed according to highly conserved region, and will not cross react with other DNAs.
- The kits are enough for 50 times of probe fluorescent quantitative PCR reaction in 20 μL system.
- The kits are available for research use only.

Developed Kits

- Chikungunya Virus (CHIKV) Probe qRT-PCR Kit
- Influenza Virus A H1N1 Probe qRT-PCR Kit
- Human Immunodeficiency Virus 1 (HIV-1) Probe qRT-PCR Kit
- HCV Probe qRT-PCR Kit
- Human Immunodeficiency Virus 2(HIV-2) Probe qRT-PCR Kit
- Dengue Virus (DENV) Probe qRT-PCR Kit

Kit Contents

Components	Package

qRT-PCR Buffer	500 μL (Blue Cap)
qRT-PCR EnzymeMix	100 μL (Red Cap)
qPCR Template Diluent	1 mL (Yellow Cap)
qRT-PCR PrimerMix (specifically designed for each virus)	100 μL (White Cap)
qRT-PCR Probe (specifically designed for each virus)	50 μL (Brown Cap)
qRT-PCR Positive Control (1×10E8 Copies/μL) (specifically designed for each virus)	50 μL (Yellow Cap)

Protocol

1. **Diluting the standard curve samples** (exemplified by 6 10-fold dilutions of 10e2-10e7 copies/μL)

   Since the concentrations of the standards are very high, the following dilutions must necessarily be performed in separate areas, and must not contaminate the samples or other components of this kit. To increase product stability and avoid the spreading of infectious diseases, no live samples are provided and only DNA fragments without infectious agents are provided as positive controls.

   1) Label 6 centrifuge tubes, designated 7, 6, 5, 4, 3, 2.
   2) Add 45 μL each of qPCR Template Diluent, preferably use filter tips.
   3) Add 5 μL of qPCR Positive Control (1×10E8 Copies/μL) in tube 7 and shake well for 1 min to obtain a standard curve sample of 1 × 10e7 copies/μL. Put on ice for further use.
   4) Replace the pipette tip and add 5 μl of 1 × 10e7 copies/μL of the positive control (obtained by diluting the sample in the last step) in tube 6 and shake well for 1 min to obtain a standard curve sample of 1 × 10e6 copies/μL. Put on ice for further use.
   5) Replace the pipette tip and add 5 μl of 1 × 10e6 copies/μL of the positive control (obtained by diluting the sample in the last step) in tube 5 and shake well for 1 min to obtain a standard curve sample of 1 × 10e5 copies/μL. Put on ice for further use.
   6) Repeat the above step until 6 dilutions of the standard curve sample are obtained. Put on ice for further use.

2. **Preparation of sample**

   7) If there are N samples, it is better to set up N + 2 extractions. One is positive control and the other is negative control. A 10,000-fold dilution of 10 μl of the positive control, plus a certain amount of water to make the total volume the same as the required volume for each preparation, can be used as a positive control. Water can be used as negative control.

8) The sample can be purified with a self-selection method and the present kit is compatible with most extraction kits on the market.

3. Probe qRT-PCR reaction

9) If quantitative analysis is needed with only 1 replicate, then label N + 9 PCR tubes with N + 2 for the N + 2 samples obtained in the previous step, 1 for the PCR negative control (using water as template) and 6 for the standard curve. If only qualitative analysis is needed with only 1 replicate, then label N + 4 PCR tubes with N + 2 for the N + 2 samples obtained in the previous step, 1 for the PCR negative control (with water as template) and 1 for the PCR positive control (with the positive control dilution from tube 4 as template). Quantitative analysis is used as the example to describe the procedure below.

10) In the labeled tubes, add each reagent as the table below (with only one replicate). Positive controls are only set after sample tubes and negative controls have already been set, and positive control samples are to be added until all other tubes had been covered and stored:

Components	Sample (N+2)	Negative Control	Standard Curve Tubes (#2-7)
qRT-PCR Buffer	10 μL	10 μL	10 μL
qRT-PCR EnzymeMix	2 μL	2 μL	2 μL
qRT-PCR Probe	1 μL	1 μL	1 μL
qRT-PCR PrimerMix	2 μL	2 μL	2 μL
N+2 DNA Samples	5 μL	-	-
Ultrapure water	-	5 μL	-
Standard Curve Tubes (#2-7)	-	-	5 μL each (#2 sample add to #2 tube，#3 sample add to #3 tube...)

11) Perform PCR with the following parameters:

Reverse Transcription	50℃	30 min
Predenaturation	94℃	3 min
qRT-PCR Reaction (40 Cycles)	94℃	15 sec
	60℃	1 min (Collect the signal of FAM channel)

12) For quantitative analysis, draw a standard curve with the log value of the positive controls' concentration as the horizontal axis and the CT value as the vertical axis. The log value of the sample concentration is extrapolated from the standard curve

with the CT value of the sample to be tested, and its concentration is extrapolated then.

13) For qualitative analysis, the negative control CT must be greater than or equal to 40. Positive controls must have logarithmic growth of fluorescence, have a typical amplification curve, and the CT value should be less than or equal to 30. Samples to be tested are considered negative if their CT value is greater than or equal to 40 and positive if less than or equal to 35. If between 35-40, repeat once. Samples are judged positive if there is a significant prominent peak in the amplification curve with CT value of less than 40 and negative otherwise.

Storage

Shipping at low temperature. -20°C for storage with a shelf life of 12 months.

Only for research and not intended for treatment of humans or animals