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Torque Teno Virus (TTV) Probe qPCR Kit

Product Number: DTK589

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of one year. The positive control should be placed separately and should not contaminate other reagents.

Component

Component	50T
2 × Probe qPCR Mix	550μL
DEPC-H_2O	1mL
Fluorescent template diluent	1mL
Human TTV qPCR Primer-Probe Mix	260μL
Human TTV qPCR Positive Control (1 × 10E8 copies/μL)	50μL

Features

This kit is used for the detection of human Torque Teno Virus (TTV). Developed based on the principle of probe-based quantitative real-time PCR, this kit has the following features:

1. Ready to use, users only need to provide a sample DNA template.
2. Primers and other components have been optimized for high sensitivity.
3. Provide positive controls to distinguish false negative samples.
4. High specificity: Primers are designed against highly conserved regions of human TTV DNA sequences and will not cross-react with DNA of other viruses.
5. It can be used for both qualitative and quantitative testing. When used for quantitative detection, the linear range should be at least 5 orders of magnitude.
6. This product is sufficient for 50 fluorescent quantitative PCR reactions using a 20μL probe system.
7. This product can only be used for scientific research.

Protocol

1. DNA extraction (sample preparation area)

1.1. If there are N samples to be extracted, it is best to set N+2 extractions, with the additional being PC (positive control for sample preparation) and NC (negative control for sample preparation). You can take 10μL of 1000 fold dilution of the positive control and add a certain amount of water to make the total volume consistent with the specified volume of the sample to be extracted, which can be used as PC. In addition, water can be used as NC.

1.2. Extract and purify sample DNA using a self selected method, and this kit is compatible with most nucleic acid extraction kits on the market.

2. Dilute standard curve sample (sample preparation area)

Due to the high concentration of positive control, the following dilution operations must be performed in a separate area to avoid contaminating the sample or other components of this kit.

2.1. Mark 6 centrifuge tubes, namely 7, 6, 5, 4, 3, and 2.

2.2. Add 45μL of fluorescent template diluent separately using a core gun tip (preferably using a core gun tip, the same below).

2.3. Add 5μL of 1 × 10E8 copy/μL positive control (provided by the reagent kit) to tube 7, shake thoroughly for 1 minute, and obtain 1 × 10E7 copy/μ L standard curve sample. Put it on ice for later use.

2.4. Change the gun head and add 5μL of 1 × 10E7 copy/μL positive control (obtained from the previous dilution) to tube 6. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E6 copy/μL. Put it on ice for later use.

2.5. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (obtained from the previous dilution) to tube 5. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E5 copy/μL. Put it on ice for later use.

2.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use. If no standard curve is required, dilute the positive control to 1 × 10E5 copies/μL.

3. Reagent Preparation (Reagent Preparation Area)

Prepare sufficient qPCR tubes (sample tube, negative control tube, positive control tube) and add the following components to each qPCR tube.

Component	N sample tubes to be tested	qPCR negative control	qPCR positive control
2 × Probe qPCR Mix	10μL each	10μL	10μL
Human TTV qPCR Primer-Probe Mix	5μL each	5μL	5μL

Transfer to the template addition area.

4. Add Template (Template Add Area)

Add 5μL template to each qPCR tube in the order: negative control (DEPC-H_2O), test sample template, Human TTV qPCR positive control. Centrifuge for 30 seconds and start amplification reaction immediately.

5. Amplification reaction (amplification and product analysis area)

Place the qPCR tube in the corresponding position of the qPCR amplification instrument sample slot for amplification. The amplification procedure is as follows:

Step	Temperature	Time
Pre denaturation	95℃	10min
qPCR reaction 45 cycles	95℃	15sec
	60℃	30sec
Signal channel	FAM channel collects fluorescence signals	

6. Result Analysis

6.1. If creating a standard curve, plot the standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the DNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and determine its concentration.

6.2. If no standard curve has been created, the results shall be judged according to the following criteria:

Valid positive control (1 × 10E5 copies/μL): Ct < 30 with obvious exponential amplification and typical S-shaped curve.

Valid negative control: Ct > 40 or no Ct value detected, without distinct exponential growth phase and plateau phase.

Sample test results: Ct < 38 with obvious exponential amplification indicates the virus is detected in the sample, result is positive; Ct > 40 or no Ct value indicates the virus is not detected in the sample, result is negative; If Ct value ranges from 38 to 40, retest the sample. If the repeated test still shows Ct value within 38–40 with obvious exponential amplification, judge as positive; otherwise negative.