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Torque teno virus (TTV) Probe qPCR Kit (with Internal Control, Lyophilized)

Product Number: DTK589L-NC

Shipping and Storage

1. This product should be stored in the dark at 2-30 ℃ and has a shelf life of 12 months.
2. After the vacuum sealed packaging of the eight row aluminum foil bag is opened, it should be stored in the dark at 2-30 ℃ and used within one week to avoid prolonged storage. The negative and positive controls after reconstitution can be stored at -15~-25 ℃ for 3 months, and below -70℃ until the expiration date of the reagent kit. Repeated freeze-thaw cycles should not exceed 3 times. The positive control should be placed separately and should not contaminate other reagents.
3. Transporting at -25-30℃ for 7 days will not affect product performance. Valid until see label.

Component

Component	48T
QPCR reaction components of human circovirus (Lyophilized)	Eight rows × 6
DEPC-H_2O	1mL
Fluorescent template diluent	1.2mL × 2
Human TTV qPCR Positive Control (Lyophilized)	Penicillin bottle × 1
Human TTV qPCR Negative Control (Lyophilized)	Penicillin bottle × 1

Note: The positive control contains the target gene fragment of human circovirus and the target gene fragment of eukaryotic reference control; The negative control contains the target gene fragment of eukaryotic reference control.

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 a positive control and set internal parameters to confirm the validity of sampling, normal extraction and amplification processes, thereby eliminating false negatives and ensuring the reliability of negative results.
4. High specificity, primers are designed based on the human circovirus gene and will not cross react with the DNA of other organisms.
5. This product is a pre packaged freeze-drying reagent that uses advanced freeze-drying technology to prepare enzymes, primer probes, and buffer systems into a loose and porous freeze-dried state in a low-temperature vacuum environment.
6. This product is sufficient for 48 fluorescence quantitative PCR reactions using a 25μL probe system.
7. This product can only be used for scientific research.

Protocol

1. DNA extraction (sample preparation area)

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. Reagent Preparation (Reagent Preparation Area)

2.1. Positive control and negative control reconstitution.

Negative control reconstitution: Take 1mL of fluorescent template diluent and add it to the negative control penicillin bottle, vortex and mix thoroughly.

Positive control reconstitution: Take 1mL of fluorescent template diluent and add it to the positive control penicillin bottle, vortex and mix thoroughly.

It is recommended to divide the negative and positive controls as needed after reconstitution to avoid repeated freeze-thaw cycles. Before each use, take it out of the refrigerator in advance, thaw it completely, and mix it thoroughly before use.

3. Add Template (Template Add Area)

3.1. Add 20μL of DEPC-H2O to each tube of the eight rows, and sequentially add 5μL of template to the qPCR tubes, in the order of human circovirus qPCR negative control, test sample template, and human circovirus qPCR positive control.

Component	48T
QPCR reaction components of human circovirus (Lyophilized)	-
DEPC-H_2O	20μL
Template	5μL

3.2. Tighten the tube cover, shake it with a vortex mixer, or lightly tap the bottom of the tube with your fingers, and repeat several times.

3.3. Observing the solution inside the tube, it is necessary to ensure that the freeze-dried powder is completely dissolved and the solution is clear and transparent (without flocs or precipitates).

3.4. If the clarification status is not reached, please repeat step 3.2; After confirming complete dissolution, centrifuge for 30 seconds and place the liquid at the bottom of the tube for qPCR amplification.

4. 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℃	3min
qPCR reaction 45 cycles	95℃	15sec
	60℃	30sec
Signal channel	FAM/HEX channel collects fluorescence signals	

The selection of fluorescent and quenching groups is shown in the following table:

Target	Fluorophore	Quencher
Human TTV	FAM	BHQ1
Reference gene	HEX	BHQ1

5. Result Analysis

5.1. Quality control

Negative control: FAM channel has no Ct value or obvious amplification curve, HEX channel Ct value<30.0;

Positive control: FAM channel Ct value ≤ 35.0, with typical amplification curve, HEX channel Ct value<30.0. The above requirements must be met simultaneously in the same experiment. If one of them is not met, the result will be considered invalid.

5.2. Sample testing results:

If the Ct value of FAM channel is < 38 and the Ct value of HEX channel is < 30.0, it indicates that the virus has been detected in the sample and the result is positive;

If the Ct value of FAM channel is ≥ 38 and the Ct value of HEX channel is < 30.0, it indicates that the virus was not detected in the sample and the result is negative;

If the Ct value of FAM channel is within the range of 38-40 and the Ct value of HEX channel is < 30.0, the sample should be retested. If the Ct value of repeated experiments is still within the range of 38-40 and there is a significant exponential increase, it is judged as positive; otherwise, it is judged as negative;

If the Ct value of HEX channel is ≥ 30.0 or there is no amplification curve, resampling and testing are required.