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Murine Parvovirus Probe-Based Quantitative PCR Kit

Product Number:DTK542

Shipping and Storage

Low temperature transportation, stored at -20 ℃, with a shelf life of one year. Positive controls need to be placed separately and should not contaminate other reagents.

Component

Component	Specifications
2 × Probe qPCR Mix	550μL
DEPC-H_2O	1mL
Fluorescent template diluent	1mL
QPCR Primer Probe Mixture for Mouse Parvovirus	260μL
Mouse parvovirus qPCR positive control (1 × 10E8 copy/μL)	50μL
User Manual	YS-P173M

Description

Parvovirus infection is one of the common pathogenic infections in rodent experimental animals. Mouse Parvovirus (MPV) has been isolated from mice. MPV is a potential pollutant in mouse spleen cell culture. Mice infected with parvovirus usually have asymptomatic clinical manifestations, but studies have shown that in experiments involving the immune system, tumors, and transplantation, in vivo and in vitro infection with MPV will seriously affect the accuracy and reliability of experimental data.

Application

This product is a specialized kit developed based on probe based fluorescence quantitative PCR technology for detecting mouse parvovirus (MPV). It has the following characteristics:

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 to distinguish false negative samples.
4. High specificity, primers are designed based on highly conserved regions of mouse parvovirus DNA sequences, and will not cross react with DNA from other biological samples.
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, and use it as PC. Additionally, use water 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. We recommend using our company's virus genome DNA extraction kit for extraction.

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, 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/μL 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)

If there are N samples to be tested, prepare N+2 qPCR tubes (N samples to be tested+1 negative control+6 positive control) and add the following components to each qPCR tube.

component	Sample tube N+2	qPCR negative control	qPCR positive control
2 × Probe qPCR Mix	10μL each	10μL	10μL
QPCR Primer Probe Mixture for Mouse Parvovirus	5μL each	5μL	5μL

Transfer to the sample preparation area.

4. Add Template (Template Add Area)

Add 5μL of template to the qPCR tube, in the order of negative control (DEPC-H_2O), test sample template, and mouse parvovirus qPCR positive control. Centrifuge for 30 seconds and immediately perform amplification reaction.

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	95℃	15sec
(45 cycles)	60℃	60sec
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:

Positive control result: Ct value<30, with significant exponential growth, showing a typical S-shaped curve.

Negative control result: Ct value>40 or no Ct value, no significant exponential growth period or plateau period.

Sample testing results: Ct value<38, with a significant exponential increase, indicating the detection of mouse parvovirus in the sample, and the result is positive; A Ct value greater than 40 or no Ct value indicates that no mouse parvovirus was detected in the sample, and the result is negative; If the Ct value is within the range of 38-40, the sample should be retested. If the Ct value of the repeated experiment 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.