MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Cytomegalovirus(CMV) qPCR detection kit

Product Number: DTK582

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-H2O	1mL
Fluorescent template diluent	1mL
Cytomegalovirus qPCR Primer Probe Mixture	160μL
Cytomegalovirus qPCR positive control (1 × 10E8 copy/μL)	50μL

Note: Different batches of reagents cannot be mixed.

Description

This kit can be used for detecting cytomegalovirus. Cytomegalovirus (CMV) is a herpesvirus DNA virus. Also known as cellular inclusion body virus, due to the enlargement of infected cells and the presence of large nuclear inclusions. Cytomegalovirus is widely distributed and can infect other animals, causing various system infections mainly in the reproductive and urinary systems, central nervous system, and liver diseases, ranging from mild asymptomatic infections to severe defects or death. Pregnant women or patients with chronic wasting diseases, low immunity, etc. should pay attention to protection.

Features

This product is a cytomegalovirus detection kit developed based on the principle of probe based fluorescence quantitative PCR. 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 positive controls to distinguish false negative samples.
4. High specificity, primers are designed based on highly conserved regions of the cytomegalovirus DNA sequence and will not cross react with DNA from 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. Suggest using the virus genome DNA extraction kit to extract DNA

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/μ L 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/μ L 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
Cytomegalovirus qPCR Primer Probe Mixture	3μL each	3μL	3μL

Transfer to the template addition area.

4. Add Template (Template Add Area)

Add 7μL of template to the qPCR tube, in the order of negative control (DEPC-H_2O), test sample template, and cytomegalovirus 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 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:

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 cytomegalovirus in the sample, and the result is positive; A Ct value greater than 40 or no Ct value indicates that no cytomegalovirus 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.