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Quantitative Detection Kit for Human Cytomegalovirus DNA (PCR - Fluorescence Probing)

Product Number: QDHC01

Shipping and Storage

1. Store below 30°C. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used HCMV PCR Master Mix should be stored at (-20±5)°C. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. Storage time should not exceed 21 days.

Components

Reagent	Components	QDHC01 48T
HCMV PCR Master Mix	Primers, probes, and reaction buffers,Mg^{2+},dNTPs,Taq DNA Polymerase	Lyophilized powder
HCMV quantitative standard 1	Recombinant plasmid containing target gene	Lyophilized powder
HCMV quantitative standard 2	Recombinant plasmid containing target gene	Lyophilized powder
HCMV quantitative standard 3	Recombinant plasmid containing target gene	Lyophilized powder
HCMV quantitative standard 4	Recombinant plasmid containing target gene	Lyophilized powder
Positive Control	Recombinant plasmid containing target gene	Lyophilized powder
Negative Control	Physiological saline	1.00mL
Redissolved Diluent	Purified water	1.40mL

Note: 1.For the specific concentrations of HCMV quantitative standard substances 1~4, please refer to the given values in the kit.

2.Do not mix reagents from different batches.

3.Material Required but Not Provided:

3.1Applicable Instrument: ABI7500, ABI 7500 Fast and other Real-time fluorescence PCR instrument with FAM, VIC channels.

3.2Vortex shaker, palm centrifuge, pipette are needed.

Description

This kit utilizes real-time fluorescent PCR detection technology, and uses specific primers and probes for HCMV to achieve quantitative detection of HCMV DNA in serum or plasma samples. The kit is provided with an internal control(IC), which can monitor whether there is PCR inhibitor in the sample to be tested by detecting whether the internal control is normal or not, so as to avoid false negative PCR.

Sample Requirements

1. Applicable sample type: serum or plasma samples.
2. Sample collection:
   2.1. **Serum:** Use a sterile syringe to draw 2mL of the subject's venous blood and inject it into a sterile centrifuge tube. Leave it at room temperature for no more than 4 hours. Centrifuge at 2000rpm for 5 minutes. Take the upper serum (be careful not to bring in red blood cells) and transfer it to another sterile centrifuge tube for use.
   2.2. **Plasma:** 2mL of venous blood is drawn with a disposable sterile syringe and injected into a glass tube containing EDTA anticoagulant. Immediately invert the glass tube slightly and mix it for 5 to 10 times to fully mix the anticoagulant and

venous blood. Centrifuge at 1500 rpm for 5 minutes; Suck the upper plasma and transfer it to a 1.5mL centrifuge tube for standby.

3. Sample storage and transportation:

The serum to be tested should be stored at 2~8°C for no more than 72 hours, and at -20°C for no more than 3 months. Samples are shipped in curling or ice-filled foam boxes.

Protocol

1. Preparation of the Reaction Mix

Take out the HCMV PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 1mL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30~60 seconds until the liquid is clear and transparent. Subpackage it into PCR reaction tubes according to 20μL/ tube.

2. Nucleic acid extraction:

This kit is not included for Nucleic Acid (NA) extraction reagent.

Commercially available extraction kits that have been shown to generate highly purified DNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted DNA is not used immediately, it should be stored below -20°C. For long-term storage, it should be stored below -80°C and avoid repeated freezing and thawing.

Note: 1)The Negative Control requires nucleic acid extraction, and the Positive Control does not require nucleic acid extraction. The Positive Control needs to be redissolved with 50μL of Redissolved Diluent and mixed well before use.

2)The HCMV quantitative standard substances 1~4 needs to be redissolved with 50μL of Redissolved Diluent and mixed well before use separately.

3. Sample adding:

Add 5μL of the sample to be tested, the extract of the Negative Control, the Positive Control and HCMV quantitative standard substances 1,2,3,4 respectively, to the PCR reaction tube. The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be centrifuged for 15s on a palm centrifuge and then delivery to the nucleic acid amplification region. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR protocols:

Place the reaction tube in the automatic fluorescent PCR instrument, set the Negative control, Positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

Select FAM channel to detect HCMV DNA, select VIC channel to detect the IC. Set the Reaction Volume per Well to 25μL.

Note: For ABI series instruments, select 'None' under 'Quencher',and select 'None' as the dye to use as the passive reference.

Recommended Setting of reaction procedure:

Step	Temperature(℃)	Time	Cycle
Pre-denaturation	95	2min	1
Denaturation	95	5s	42
Annealing, extension, and fluorescence collection	60	35s	

5. Result analysis

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3~15, the End value can be set at 5~20, the amplification curve of the Negative Control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis; make the parameters meet the requirements in the following '6. Quality control', and then go to the

Plate window to record the Ct value.

6. **Quality control**

Negative control: FAM and VIC detection channels have no amplification curves.

Positive control: The detection concentration of FAM channel is between 1.00×105~1.00×106 IU/mL, and the Ct value of VIC channel ≤30.00.

HCMV quantitative standard substances 1~4:FAM channel has obvious amplification curve, and the standard curve correlation coefficient |r|≥0.98.

The above requirements must be met at the same time in the same experiment; otherwise this experiment is invalid and needs tobe repeated.

Positive Judgment Value

Through the study of the reference value, the minimum detection limit of this kit for HCMV was determined to be 200 IU/mL, and the Ct positive judgment value of VIC was 35.00.

Explanation of Test Results

1. For samples with measured values between 1.00×10^3IU/mL and 1.00×10^8IU/mL, report the corresponding determination results.
2. For samples with measured value >1.00×10^8 IU/mL, report >1.00×10^8 IU/mL. If accurate quantitative results are required, the extracted sample can be diluted to within the linear range and tested.
3. For samples with measured value ≥200IU/mL and less than 1.00×10^3IU/mL, the Internal Control detection is positive and Ct value≤35.00, indicating that the viral load is low, and the measurement results are for reference only.
4. For samples with measured value <200IU/mL, and the Internal Control detection is positive and Ct value≤35.00, it was reported as lower than the detection limit of the kit. If the Internal Control is abnormal, the test result of this sample is invalid, and the test is repeated for this sample.

Limitations

1. The test results are for clinical reference only, and the clinical diagnosis and treatment of patients should be comprehensively considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment response.
2. The sample test results are related to the quality of sample collection, processing, transportation and storage, and any mistakesmay result in inaccurate results. False-positive results may occur if cross-contamination is not controlled during sample processing.
3. The detection of this kit is for the conserved region of the pathogen, but it does not rule out that the gene mutation of thepathogen during the epidemic may lead to false negative results.
4. This kit is limited to the sample types and applicable models specified in this manual. Validation should be performed before using other sample types and models.
5. A negative test result only means that the viral load in the specimen is lower than the detection limit of this kit, but does not rule out the possibility of infection with HCMV.

Performance Parameters

1. Limit of Detection: The minimum detection limit of this reagent for HCMV is 200 IU/mL, the lowest limit of quantification is 1.00×10^3 IU/mL.
2. Linear range: 1.00×10^3IU/mL~1.00×10^8 IU/mL.
3. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) value of detected concentration logarithm is ≤ 5.00%.
4. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise reference is 100%, and the compliance rate of positive reference products is 100%.

5. Analysis of Specificity:
   5.1. Cross-reaction: There is no cross-reaction with Epstein Barr virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, syphilis, herpes simplex virus type 1/2.
   5.2. Interfering substances: When the concentrations of interferon α, penicillin G and acyclovir are 1×10^6U/L, 2×10^6U/L and 66.6μmol/L respectively, the concentrations of bilirubin, free hemoglobin, triglyceride, total IgG, EDTA and sodium citrate in the sample are 300μmol/L,100mg/L,5mmol/L,80g/L,1.5g/L,6g/L, there is no interference with the test results of the kit.

Notes

1. This kit is for in vitro detection only. Please read the kit instructions carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and carry out quality control for each experiment.
3. The entire experimental operation process and the hardware and software facilities of the PCR laboratory should comply with the requirements of the Ministry of Health. The operator must be trained in the use of real-time fluorescent PCR instrument.
4. The experimental process should be carried out in different areas (reagent preparation area, sample preparation area, amplification and product analysis area). Laboratory consumables (e.g. centrifuge tubes, suction tubes) should have proper cleaning and quality control procedures to avoid false negative results that caused by amplification reaction inhibitors.
5. In order to avoid any potential biological hazards from the sample, all test samples should be regarded as an infectious substance.
6. Samples to be tested must be fully thawed and mixed at room temperature before use.