Human Cytomegalovirus (CMV) Detection Kit (Real-time PCR)

Instruction for Use (V1.0)

[REF] UBP-S00850H

[Specification] 50 tests/kit

[Research Use Only]

Human cytomegalovirus (CMV) is a member with the largest genome in the herpes virus family and can encode more than 200 proteins. CMV is narrowly restricted in its host range to humans, and there is still no animal model of its infection. HCMV has a slow and long replication cycle to form an intranuclear inclusion body, and trigger the production of perinuclear and cytoplasmic inclusion bodies and cell swelling (giant cells), hence the name. According to the heterogeneity of its genome and phenotype, CMV can be divided into a variety of strains, among which there are certain antigenic variations, which, however, are of no significance.

CMV infection is a systemic infection, which involves multiple organs, has complex and diverse symptoms, is mostly silent, and can cause a few patients to develop multiple-organ lesions including retinitis, hepatitis, pneumonia, encephalitis, colitis, monocytosis, and thrombocytopenic purpura. CMV infection is very common and appears to spread worldwide. It is highly prevalent in the population, with incidence rates of 45-50% and more than 90% in developed and developing countries, respectively [1]. CMV can lay dormant in the body for a long time. Once the body's immunity is weakened, the virus will be activated to cause diseases, especially recurrent infections in leukemia patients and transplant patients, and can cause transplanted organ necrosis and endanger the life of patients in severe cases [2-3]. In addition to stillbirth, miscarriage and premature delivery via intrauterine infection, cytomegalovirus can also cause congenital malformations, so CMV infection is able to affect prenatal and postnatal care and population quality [4].

This kit is used for qualitative determination of nucleic acids in samples including blood from patients with suspected CMV infection. The test results are for research use only and should be closely combined with data for final conclusion.

[Test Principle]

This kit uses PCR combined with fluorescent probes. It contains specific primers and probes for fluorescence detection, which are designed to target the highly conserved region of CMV. The fluorescent probes are labeled with FAM at the 5'-end and the quencher BHQ1 at the 3'-end. At the same time, the kit contains internal references and corresponding specific primers and probes, of which the probes are labeled with the fluorophore VIC (HEX) at the 5'-end and the quencher BHQ1 at the 3'-end. During PCR amplification, CMV-specific primers and probes bind to target sequences, and complete synchronization of PCR product formation and fluorescence signal accumulation is achieved based on the polymerase activity and 5'-3' exonuclease activity of the Taq DNA polymerase, thereby realizing the qualitative detection of target nucleic acids in a sample.

[Kit Contents]

No.	Name	Specification	Description
1	CMV PCR Reaction Solution	1 mL/vial×1	A mixture in certain proportions of CMV- specific primers and probes, primers and probes compatible with internal references, and hot- start DNA polymerase
2	CMV Positive control	250 μL/vial×1	CMV plasmids and recombinant plasmids of internal references
3	CMV Blank control	250 μL/vial×1	RNase-free and DNase-free water

Note: The contents of the kit in different batches are not interchangeable.

Reagents required but not provided: Viral Genomic DNA/RNA Extraction Kit manufactured by Ultrassay or QIAamp DNA Mini Kit (Cat. no.51304) manufactured by QIAGEN.

Materials required but not provided: 1.5 mL RNase-free and DNase-free centrifuge tubes, RNase-free and DNase-free tips, benchtop centrifuge, benchtop vortex mixer.

[Storage Conditions and Shelf Life]

This kit should be stored below -18°C protected from light, and its shelf life is 12 months. It shall not be subjected to repeated freezing and thawing for more than 4 cycles, and it can be stored stably for 5 days when it is transported below -18°C under dark conditions.

[Applicable Equipment]

ABI 7500, ABI Stepone, Ultrassay XP96 Real Time qPCR System.

[Acceptable Specimens]

1. Sample collection

Freshly collected human serum, plasma or urine samples from patients with suspected CMV infection

2. Storage

The sample to be tested is stored below -70°C after collection, and repeated thawing and freezing is prohibited.

3. Transportation

Samples are transported using airtight foam boxes with dry ice.

[Test Procedures]

1. Preparation of reagents

1.1 Place the CMA PCR Reaction Solution at room temperature until it is fully dissolved, then shake well, and centrifuge it briefly.

1.2 Calculate the number (N) of samples to be prepared (N = number of samples + 1 tube of the blank control + 1 tube of the positive control). According the number (N) of samples required, add 20 μL of the CMA PCR reaction solution to a PCR tube, press the tube cap tightly and label the tube, then transfer it to the sample processing area.

2. Sample processing

It is recommended to extract DNA from a sample in strict accordance with the instructions for use of Viral Genomic DNA/RNA Extraction Kit (DP315) manufactured by QIAamp DNA Mini Kit (Cat. no.51304) manufactured by QIAGEN (no processing or extraction is required for the positive control or the blank control).

3. Sample loading

Add 10 μL of the test sample that has been treated as per Step 2, 10 μL of the positive control and 10 μL of the blank control to the CMA nucleic acid reaction solution aliquoted in Step 1, press the tube cap tightly, and centrifuge the tube briefly. Put the tube into the PCR instrument, and record the loading sequence.

4. PCR amplification

Channel setting:

1) Select the FAM channel (Reporter: FAM, Quencher: None) to detect CMV DNA;

2) Select the VIC (HEX) channel (Reporter: VIC (HEX), Quencher: None) to test internal references;

3) Reference Dye: Select None (only applicable to ABI instruments)

4) Sample Volume is 30. For specific detection channel settings, please refer to the instructions for use of each instrument.

5) Setting of PCR conditions is shown in the following table:

Step	No. of Cycles	Temperature	Duration	Fluorescence Signal Collection

1	1	50℃	2 mins	NO
2	1	95℃	10 min	NO
3	40	95℃	15 secs	NO
		60℃	30 secs	Yes

5. Result analysis

5.1 Baseline and threshold setting for ABI 7500 Fluorescence-based qPCR Instrument

Baseline setting: When setting the baseline for a certain channel, first select the sample to be tested with the smallest Ct value, change the option "☑Auto Baseline" to "☐ Auto Baseline", then select ☑Baseline Start, and manually adjust the baseline end point to be less than the minimum Ct value of the sample.

Threshold setting: The threshold for each channel should be set separately. When setting the threshold line for a certain channel, first select the blank control to be tested, remove the checked automatic threshold line, change the option "☑Auto" to "☐ Auto", and then manually adjust the threshold line to just exceed the peak of the normal blank control's amplification curve (irregular noise line) in the FAM channel and VIC/HEX channel.

6. Quality control:

1). Blank control: no obvious amplification curve in the FAM channel, and a Ct value of 0 or a Ct value > 35 in the VIC (HEX) channel.

2). Positive control: a Ct value ≤ 30 in the FAM channel, and a Ct value ≤ 30 in the VIC (HEX) channel.

The above requirements must be met at the same time in the same test, otherwise, such test is invalid and a re-test is required.

[Reference Range]

The Ct value for the kit is analyzed using the ROC curve analysis and percentile. It is determined that the positive judgment value for the kit for the detection of CMV is a Ct value ≤ 38; the positive judgment value in the internal reference is a Ct value < 35.

[Explanation of Test Results]

1. If the FAM channel has no obvious amplification curve and a Ct value ≤ 38, then it is determined that the test sample is negative for CMV.

2. If the FAM channel has no amplification signal, and the VIC/HEX channel has an amplification curve with a Ct value ≤ 35, then it is determined that the test sample is negative for CMV.

3. If the FAM channel has an obvious amplification curve with 38 < Ct < 40 and the VIC (HEX) channel has a Ct value ≤ 35, then a re-test is required. If the re-test shows 38 < Ct < 40 in the FAM channel and a Ct value ≤ 35 in the VIC (HEX) channel, then a positive test result will be reported; if the re-test shows no amplification curve and a Ct value ≤ 35 in the VIC (HEX) channel, then the test result is considered negative.

4. If the test sample shows no amplification curve in the FAM channel and a Ct value > 35 or no Ct value in the VIC (HEX) channel, then it is recommended to re-extract a DNA sample for testing.

[Limitations of Test Method]

1. Any of unreasonable processes of sample collection, transportation and handling/processing may result in inaccurate test results.

2. Contamination of amplification products and cross-contamination between samples subjected to nucleic acid extraction are prone to cause false positive results. Therefore, laboratories should be equipped with equipment in strict accordance with the Laboratory SOP for Gene Amplification, and operators should strictly follow the Instructions for Use.

3. A negative test does not mean that the patient is not subjected to HCMV infection. A final conclusion must be made in conjunction with other data. A negative test may be caused by: ① unreasonable processes of sample collection, transportation and handling/processing, or a low nucleic acid content in samples; ② variations in target sequences or sequence changes caused by other reasons; ③ other interference factors that have not been verified or PCR inhibitors that prevent the amplification of nucleic acids.

[Product Performance]

1. The kit is intact in appearance with clear transparent liquid components free of insoluble matter.

2. For the blank control in the kit, there is no obvious amplification curve in the FAM channel, and there is a Ct value of 0 or a Ct value > 35 in the VIC (HEX) channel. For the positive control, there is a Ct value ≤ 30 in the FAM channel, and a Ct value ≤ 30 in the VIC (HEX) channel.

3. The results from the inhouse positive and negative reference standards tested by the kit should comply with the requirements.

4. Among the 20 replicate assays by the kit on the inhouse LOD reference standard: in the FAM channel of the CMA PCR reaction solution the virus should be detected by at least 18 assays, and in the VIC channel, the virus should be detected by all the 20 assays.

5. Repeatability: Each repeatability reference standard is subjected to 10 replicate assays by the kit, and the CV should be ≤ 5.0%. 6. Specificity: All test results are negative for hepatitis B virus, hepatitis C virus, human papilloma virus, herpes simplex virus type 1, herpes simplex virus type 2, normal human serum samples, etc.

[Precautions]

1. This kit is used for qualitative detection of CMV nucleic acids in pharyngeal swabs and sputum samples. Please read the Instructions for Use carefully before testing.

2. The results obtained by this kit will be affected by factors including the source and quality of a sample itself, sample transportation conditions and sample pretreatment, as well as restricted by the quality of extracted DNA, working status of fluorescence-based qPCR instruments, operating environments and the limitations of current molecular biology technology, which may result in false-positive or false-negative test results. The user is required to understand the potential errors and accuracy limitations that can arise during testing.

3. The kit should be transported and stored at a low temperature. Before use, all reagents in the kit should be completely thawed and shaken evenly, and then centrifuged briefly. Avoid unnecessary repeated thawing and freezing.

4. All reagents in the kit have been specially prepared for the above detection. Replacement of any reagent in the kit may affect its effect. The components of the kit in different batches cannot be mixed with each other.

5. When carrying out a test, perform different operations strictly according to different areas:

Area1: Reagent preparation area — to prepare the reagents needed for amplification;

Area2: Sample processing area — to handle samples to be tested and controls (reference standards);

Area3: Loading area — to add a sample and reference standard to a corresponding reagent;

Area4: Testing area — to perform a PCR amplification assay.

Testing should be performed in strict accordance with relevant laboratory SOPs for gene amplification testing issued by administrative departments.

Items in each area are for exclusive use and shall not be cross-used to avoid contamination; please clean the workbench after testing. The lysates from samples stored below −18°C or −70°C should be thawed at room temperature before loading, and used after brief centrifugation.

6. During testing, pay attention to preventing the contamination of the reagents by exogenous nucleic acids, and pay attention

to adding the DNA sample followed by the positive control. It is recommended to use separate, dedicated pipettes and pipette tips when preparing reagents and adding templates.

7. The reaction tube containing the reaction solution should be capped and put into an airtight bag before transferring to the sample processing area.

8. When aliquoting the reaction solution, avoid air bubbles as far as possible. Before loading, check whether each reaction tube is tightly capped to prevent the leakage of fluorescent substances, which may contaminate the instrument.

9. When a sample is added, it should be completely dropped into the reaction solution, that is, there should be no residue adhering to the tube wall; tighten the cap as soon as possible.

10. After amplification is completed, take out the reaction tube immediately, seal it in a dedicated plastic bag, and discard it in a designated place.

11. All centrifuge tubes and tips used during testing must be free of RNase and DNase. All centrifuge tubes and tips used during testing must be treated in a harmless manner. All pipette tips used during testing should be directly put into a tank containing 84 disinfectant for disposal of waste, and sterilized together with other waste items before discarding them.

12. The workbench and various experimental supplies should be regularly disinfected with 75% alcohol or ultraviolet light.

13. All chemicals are potentially dangerous. During operation, please wear appropriate laboratory overalls and take protective measures including disposable gloves.

[References]

[1]. Walker SP, Palma-Dias R, Wood EM, et al. Ytomegalovirus in pregnancy: to screen or not to screen[J]. BMC Pregnancy Childbirth, 2013, 13:96.

[2]. Popovic M, Smiljanic K, Dobutovic B, et al. Human cytomegalovirus infection and atherothrombosis[J]. J Thromb Thrombolysis, 2012, 33(2):160-172.

[3]. Huang ZR, Yu LP, Yang XC, et al. Human cytomegalovirus linked to stroke in a Chinese population[J]. CNS NeurosciTher, 2012, 18(6):457-460.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
	Number of Tests		Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
	Lot Number		Reference Number
			Any warnings and/or precaution to take

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