MBPCR279 Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit

Description

Human cytomegalovirus (CMV) is a herpesvirus with a prevalence of about 100% in both Africa and Asia, and 80% in Europe and North America. Human cytomegalovirus is a double-stranded DNA virus with a genome of nearly 240 kb. It is a member of the family Herpesviridae and genus Cytomegalovirus. CMV is classified as a β-herpesvirus (HHV-5) and is considered to be the largest herpesvirus to infect humans. The primary CMV infection similar to other herpesviruses remains dormant within the healthy individuals however viral reactivation can occur in response to compromised immune system. Immunocompromised patients, particularly transplant recipients, HIV-infected or cancer patients, blood transfusion patients are regarded as high-risk populations for CMV. Transmission of CMV can occur in numerous ways: via blood products (transfusions, organ transplantation), breastfeeding, viral shedding in close-contact settings, perinatally, and sexual transmission. CMV infected pregnant mother can pass the infection to fetus causing congenital cytomegalovirus infection leading to morbidity and even death. CMV disease can mimic a range of different manifestations and pose significant diagnostic challenges, leading to late or inaccurate diagnosis and adverse health outcomes. Hence high-risk population and pregnant ladies are usually subjected to CMV infection testing. Among several modalities available for CMV diagnosis, quantitative NAAT-based CMV viral load testing has become a major tool for diagnosing active disease, initiating preemptive therapy, monitoring response to antiviral therapy, and signaling the risk of clinical relapse or antiviral resistance.

NOTE: HiMedia's Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit is for *in vitro* use only.

Intended Use

Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The kit is recommended for detection and quantification of CMV specific DNA (100%) in human plasma and urine samples.

Product Description:

Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit is based on real-time PCR technology for the detection and quantitation of Cytomegalovirus (CMV) specific gene. The kit contains primer-probe mixture specific for detection of CMV DNA. In addition, the kit can identify the DNA extraction efficiency and ensure successful PCR reaction with the help of an endogenous internal control. The quantitative standards (CMV quantitative standards QS1-QS4) are supplied in the kit which allow the determination of viral DNA load. The assay principle is based on hydrolysis probe chemistry which confers higher specificity and sensitivity.

Positive control (or Quantitative standards)

This is a control reaction used to test for the presence of inhibitors in the sample or the efficiency of the polymerase chain reaction itself using a pre-dispensed DNA sequence and the primer set that detects it. It is usually used to ensure proper and intended functioning of all the reagents and is recommended to be used in every run to assess optimal performance.

Endogenous Internal control

This is a control sequence that should amplify in all clinical samples which indicates the presence of sufficient amount of DNA indicating the specimen is of acceptable quality. An internal control also allows to check for optimal sample collection, DNA extraction, possible PCR inhibition or reagent failure.

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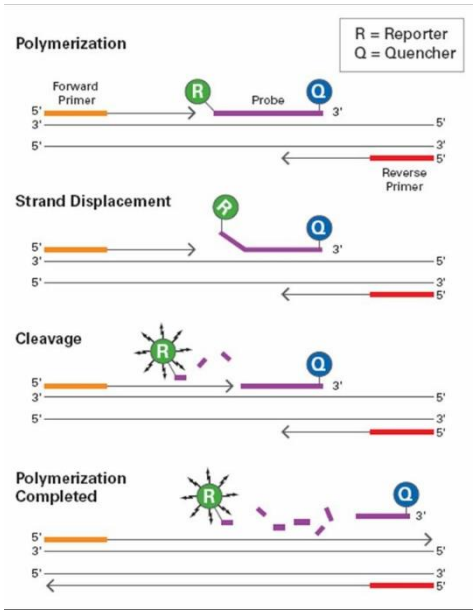
Negative Template Control

A negative template control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target DNA. In this reaction, Nuclease free water is used as the template. It is recommended to have a minimum of one reaction of negative template control per run.

Principle

Real-Time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit is designed to specifically detect **CMV DNA in FAM channel** with **Internal Control (IC) in ROX channel**.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5’ and 3’ end of the probe respectively

Strand displacement: When the probe is intact, the reporter dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerase cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Molecular and Technology features:

- Sensitive and specific
- Fast and reliable results within 65 minutes
- Includes quantitative standards for calculation of viral DNA load
- Open system – Compatible with 4-channel and 5-channel qPCR cyclers
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Types of Specimens and storage:

The internal validation of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit was performed using DNA extracted from human EDTA plasma and urine samples. Other sample materials are not validated. Therefore, we recommend the use of urine and human EDTA plasma samples for detection of CMV using Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit. The DNA should be extracted using a standard viral DNA extraction kit. After collection, clinical specimens can be kept refrigerated at 4 °C if shipped in less than 72 hours of collection; otherwise specimen should be kept frozen at -20°C. After extraction, store the extracted DNA samples at -20°C for short period storage and -70°C or -80°C for long period storage.

Specimen Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided (stable for 5 freeze-thaw cycles), as this may reduce the sensitivity of the assay. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

Kit Contents

The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)*		
		25R	50R	100R
CMV Master Mix	DS1897	135 µL	270 µL	530 µL
CMV Primer-Probe Mix	DS1898	27 µL	54 µL	106 µL
CMV-QS1 (5 x10^6 IU/µL)	DS2314	54 µL	54 µL	106 µL
CMV-QS2 (5 x10^5 IU/µL)	DS2316	54 µL	54 µL	106 µL
CMV-QS3 (5 x10^4 IU/µL)	DS2317	54 µL	54 µL	106 µL
CMV-QS4 (5 x10^3 IU/µL)	DS2319	54 µL	54 µL	106 µL
Molecular Biology Grade Water	ML065	162 µL	270 µL	530 µL

Materials needed but not provided

- Appropriate real-time PCR instrument.
- Appropriate nucleic acid extraction system or kit.
- Centrifuge with a rotor for 1.5ml - 2 ml reaction tubes.
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates.
- Vortex mixer.
- PCR tubes (0.1ml or 0.2ml) or 96 well reaction plates with corresponding (optical) closing material or lid.
- Pipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL).
- Pipette tips with filters (As per pipette capacity).
- Powder-free gloves (disposable).

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012

Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
TabSpin™ Microcentrifuge	LA1089/LA1090
HiPer® Mini Plate Centrifuge	LA1099
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96, Insta NX® Mag96^{plus}	LA1097, MBLA026
Extraction Kits	
HiPurA® Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification	MB582MPF16
HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag32]	MB582MPF-32
HiPurA® Prefilled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag96]	MB582MPF-96
HiPurA® Viral DNA/RNA Purification Kit	MB582
HiPurA® Viral DNA Purification Kit	MB575
Insta NX® Viral DNA Purification Kit	MBIN015
Tubes, plates, and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips,100µl Max capacity 100 µL	LA1104A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1 mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18
1.5 ml nuclease free Micro centrifuge tubes	PW146

Kit compatibility with Real-Time PCR Systems

Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1 (CMV)	Dye 2 (IC)
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	ROX
QuantStudio™ 3 and 5	Applied Biosystems	FAM	ROX
Applied Biosystems 7500	Applied Biosystems	FAM	ROX
BioRad CFX Opus 96/CFX96	Bio-Rad Laboratories, Inc.	FAM	ROX
Rotor-Gene® Q/QIAquant	QIAGEN	Green	Orange
Roche LightCycler® 96	Roche	FAM	ROX
AriaMx	Agilent	FAM	ROX
Alta RT-96/48	Athenese-Dx Private Limited	FAM	ROX
qTOWER^3 auto	Analytik Jena	FAM	ROX

Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufactureŕs instructions and recommendations

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Clear surfaces and working areas with HiMedia's DNA Kil™ solution (ML221).

Protocol for PCR Reaction Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), calculate the volume of the components to be added as N X (volume of "1X")
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume for "1X" (One Reaction)
Preparation of PCR Reaction Mix		
CMV Master Mix	DS1897	5 µL
CMV Primer-Probe Mix	DS1898	1 µL
Molecular Biology Grade Water	ML065	4 µL
Total PCR Reaction Mix	**-**	**10 µL**
Template addition		
Quantitative Standards/Purified Viral DNA	**-**	10 µL
Total reaction volume	**-**	**20 µL**

4. Aliquot 10 µL of PCR reaction mix into respective labeled 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add 10 µL of extracted DNA of test specimen into the plate/strip to respective wells.
6. For positive and no template control, purified template Viral DNA is replaced by quantitative standards and by nuclease free water respectively.
7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

Step	Temperature	Time	Sampling	Cycles
1	95°C	10 minutes	---	1
2	95°C	10 seconds	---	45
3	60°C	25 seconds	Yes	

Selection of channel

Target	Dye	Quencher[#]
CMV	FAM	None
IC	ROX/Texas Red	None

[$]**Passive Reference Dye:** Select "None"
([#,$]Thermo Fisher's QuantStudio™ 5 Real-Time PCR System)

Data Analysis

The following conditions should be met for a valid diagnostic test:

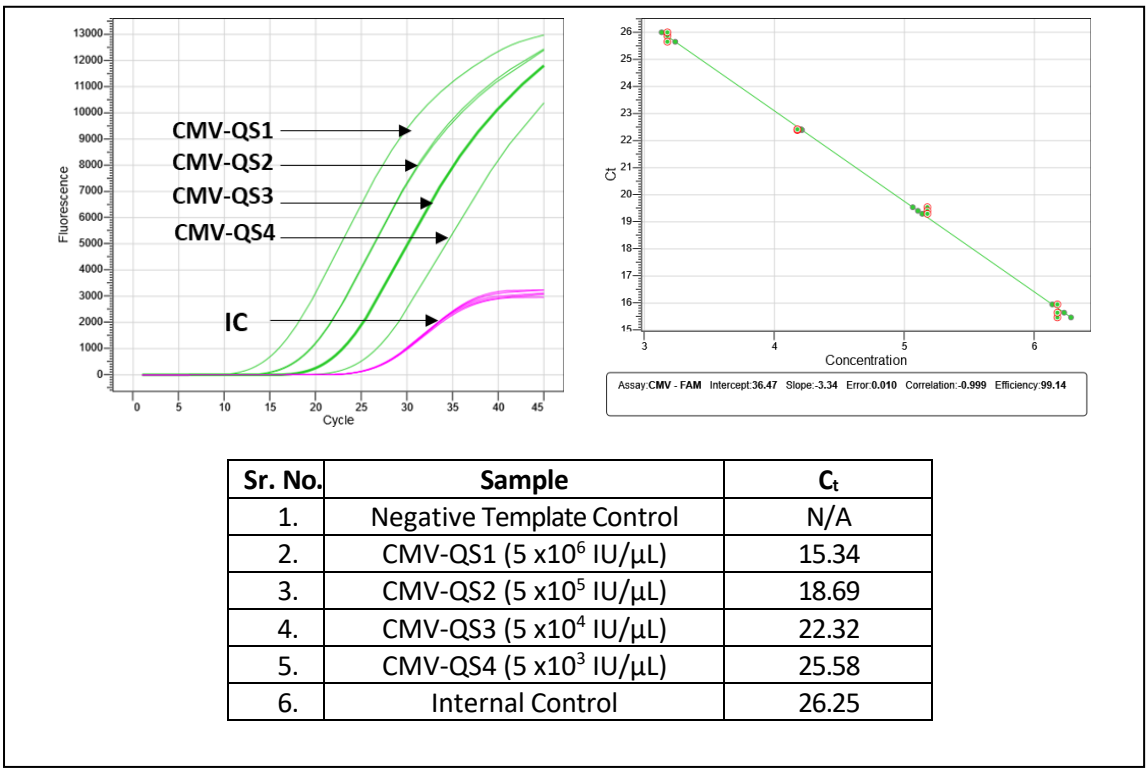
Control	Detection channel	
	FAM (CMV)	ROX (Internal Control)
CMV-QS1 (5 x10^6 IU/µL)	+	+
CMV-QS2 (5 x10^5 IU/µL)	+	+
CMV-QS3 (5 x10^4 IU/µL)	+	+
CMV-QS4 (5 x10^3 IU/µL)	+	+
Negative Template Control	-	-

Standard Curve Analysis

Interpret the values for unknown samples, only if the following conditions are met:

1. R^2 >0.98
2. Slope of the quantitative standards is between -3.1 to -3.7
3. PCR efficiency is between 85%-115%
4. No amplification in FAM channel of negative template control

Amplification Plots



Sr. No.	Sample	C_t
1.	Negative Template Control	N/A
2.	CMV-QS1 (5 x10^6 IU/µL)	15.34
3.	CMV-QS2 (5 x10^5 IU/µL)	18.69
4.	CMV-QS3 (5 x10^4 IU/µL)	22.32
5.	CMV-QS4 (5 x10^3 IU/µL)	25.58
6.	Internal Control	26.25

Image representing amplification plot and standard curve of CMV quantitative standards with Ct values using HiMedia's Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit on InstaQ 96 series of instrument (Ct values provided in table are for representation only).

HiMedia's Hi-PCR® Cytomegalovirus (CMV) Probe PCR kit can also be used for qualitative detection of CMV by using CMV quantitative standards 2 (CMV-QS2) with expected Ct values 18.69 ± 2.0.

Data Interpretation:
When all the standards fulfill the stated requirements, interpret the results of the specimen as follows:

Detection Channel		Result Interpretation
FAM (CMV)	**ROX (Internal Control)**	
+ (Ct cut off ≤ 39)	+/-*	CMV specific DNA detected
-	+	CMV specific DNA is not detected. Sample does not contain detectable amounts of CMV specific DNA.
-	-	PCR Inhibition or reagent failure. Retest the sample.

*Detection of the IC in the ROX channel is not required for the positive results in the FAM channel. Presence of high CMV DNA load and/or PCR inhibitors in the original sample can lead to reduced or absence of internal control signal.

Note:
We recommend that the test results must be interpreted by an expert. Clinical correlation along with patient history is necessary to determine patient infection status.

Threshold value set up:
Set the appropriate threshold values based on the real-time PCR instrument. Minimum threshold value set up of the target gene (CMV) for some of the commonly used thermal cyclers are as follows:

Sr. No	Real-Time PCR instrument	Minimum Threshold
1.	InstaQ96	Auto threshold
2.	Applied Biosystems QuantStudio 3/5	50000-55000
3.	Bio-Rad CFX-96	150-250

The threshold value varies between different instruments depending upon the age, model and the calibration. The threshold values must be validated for the specific instruments. Please contact our technical team for any queries.

Use following formula to convert concentration into IU/mL:
The quantitation standards given in the kit are defined as IU/µL. As the initial sample volume and elution volume will be different during isolation of viral DNA, the following formula should be used to convert the concentration values determined using the standard curve into IU/mL.

$$\text{Result (IU/mL)} = \frac{\text{Concentration of sample (IU/µL) x Elution Volume (µL)}}{\text{Original Sample Volume (mL)}}$$

To convert results in copies/ml use the following formula

Copies/mL = IU/mL X 1.12 (Conversion Factor)

Performance Characteristics
Analytical sensitivity
The analytical sensitivity of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR kit is defined as the concentration of CMV DNA molecules that can be detected with a positivity rate of **≥ 95%**.
The analytical detection limit in consideration with nucleic acid extraction was determined using CMV spiked plasma specimens in combination with a particular extraction method. In addition, the analytical detection limit independent of extraction was determined using a standard CMV DNA of known concentration and Human herpesvirus 5; HCMV Strain AD-169 (ATCC- VR-538DQ).

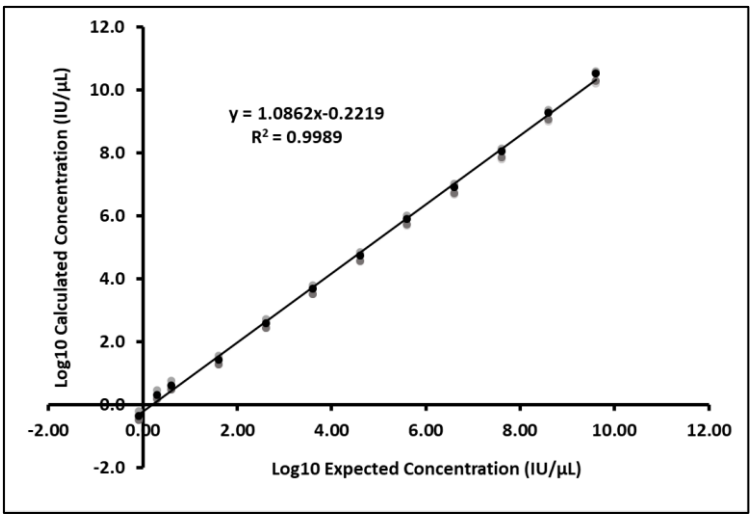
The analytical sensitivity in consideration with nucleic acid extraction was determined using a dilution series of the 1st International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) from 5 x 10^5 IU/mL to 100 IU/mL spiked into CMV negative EDTA plasma. Experiments were carried out in triplicate independent extractions (Sample volume: 200 µL, elution volume: 60 µL), followed by triplicate real-time PCR reactions for each extraction for three to four consecutive days on InstaQ 96®Plus real time PCR system. The analytical detection limit in consideration of the purification of Hi-PCR® Cytomegalovirus (CMV) Probe PCR kit is **160 IU/mL**.

In addition, the analytical detection limit independent of extraction was determined using standard CMV DNA of known concentration and Human herpesvirus 5; HCMV Strain AD-169 (ATCC- VR-538DQ). A dilution series of the standard CMV DNA was set up from 5 x 10^9 IU/µL to 0.5 IU/µL and analyzed in triplicates with the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit. The analytical sensitivity of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit based on standard CMV DNA is **1 IU/µL**. The analytical sensitivity with Human herpesvirus 5; HCMV Strain AD-169 (ATCC- VR-538DQ) was determined to be **≈1 copy/µL.** The preliminary analytical sensitivity was determined by testing a 10-fold dilution series in triplicates per concentration, and then confirmed with 20 replicates of the concentration determined to be the analytical sensitivity of the kit. The sensitivity analysis of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit was carried out on HiMedia's InstaQ 96 series, Biorad's CFX Series and Thermo Fisher's QuantStudio™ 5 Real-Time PCR System.

Linear Range

The linear range in consideration of the purification of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit was determined by analyzing a logarithmic dilution series of the 1st International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) and the HiMedia's CMV Quantitative Standard Set. Each dilution was extracted in independent triplicates (Sample volume: 200 µL, elution volume: 60 µL) and each extraction was further tested in triplicates using Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit and analyzed on InstaQ96 series of instruments. The linear range of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit in consideration of the purification has been determined to cover concentration from **5 X 10^5 IU/mL to 160 IU/mL.**

The linear range (analytical measurement) of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit was determined by analyzing a dilution series of standard CMV DNA from 5 x 10^9 IU/µL to 0.5 IU/µL. The dilution series has been calibrated against the 1st International Standard for Human Cytomegalovirus (HCMV). Each dilution of CMV quantitative standard for concentration ranging from 5 x 10^9 IU/µL to 2.5 IU/µL was tested in triplicates; the concentration 1 IU/µL was tested in 20 replicates using the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit. The linear range of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit has been determined to cover concentrations from **5 x 10^9 IU/µL to 1.0 IU/µL.**



Linear Range of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit: The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ expected concentrations when using CMV DNA.

Analytical Specificity

Inclusivity – In silico

The analytical specificity of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR kit was ensured by performing *in silico* analysis of the CMV primers and probes using NCBI BLAST and optimizing the stringent PCR conditions. The CMV primers and probes were checked for possible homologies to all sequences published in NCBI database using multiple sequence alignment tools. The detectability of all relevant strains has thus been ensured.

Analytical Reactivity

The analytical reactivity of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR kit was verified by wet lab testing of the oligonucleotides (primers and probes) against commercially available standard - Human herpesvirus 5; HCMV Strain AD-169 (ATCC- VR-538DQ).

Cross-Reactivity

Wet lab testing was performed against the genomic or synthetic DNA of the pathogens (from ATCC) available in the laboratory on Thermo Fisher's QuantStudio™ 5 Real-Time PCR System for any potential cross-reactivity. None of the tested pathogen in the below mentioned table showed any reactivity to the primers- probes of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit.

Human immunodeficiency virus 1 (HIV-1) (VR- 3245SD)	Human papillomavirus 16 (VR-3240SD)
Hepatitis A virus (VR-3257SD)	Human papillomavirus 18 (VR-3241SD)
Hepatitis B virus (VR-3232SD)	Human adenovirus 1 strain Adenoid 71 (VR-1DQ)
Hepatitis C virus (VR-3233SD)	Plasmodium falciparum strain 3D7 (PRA-405)
Staphylococcus aureus subsp. aureus F-182 (43300DQ)	Plasmodium vivax DNA (PRA-3004SD)
Dengue virus (1-4) (VR-3228SD, VR-3229SD, VR-3230SD, VR-3231SD)	Plasmodium malariae (3001SD)
Human herpesvirus 3 (HHV-3) (VR-1367DQ)	Candida albicans strain SC5314 (MYA-2876DQ)
Human gamma herpesvirus 4 (VR-3247SD)	JC Polyomavirus (VR-1583DQ)
Human coronavirus 229E (VR-740D)	Aspergillus niger strain A1144 3528.7 (1015DQ)
Enterovirus (VR-1826DQ)	Giardia intestinalis (30888D)
Escherichia coli (10798DQ)	Pseudomonas aeruginosa (47085DQ)

Cross-Reactivity *In silico* Analysis

The primer and probe sequences of the oligonucleotides (primers and probes) in the Hi-PCR® Cytomegalovirus (CMV) Probe PCR kit were subjected to a BLAST (Basic Local Alignment Search Tool) analysis against the pathogens shown in the below table. These pathogens included viruses related to CMV, viruses causing similar symptoms as an infection with CMV and viruses likely to be present in patients suffering from a CMV infection. No significant cross- reactivity was noted for all the subject sequences when evaluated by *in-silico* Blast analysis.

West Nile virus (taxid:11082)	Human herpesvirus 7 (taxid: 57278)
Human herpesvirus 1 (taxid: 10298)	Human herpesvirus 8 (taxid: 435895 and 868565)
Human herpesvirus 2 (taxid: 10314, 10313, 213997 and 213998)	Human parvovirus B19 (taxid:10798)
Human herpesvirus 6 (taxid:36351)	

Extraction kit compatibility:

The extraction kits and systems like HiMedia viral nucleic acid purification kits; MB582MPF16, MB582MPF-32 and QIAamp MinElute Virus Spin Kit are validated with the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit. Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit must be validated by the user.

Warning
Not for Medicinal Use.
Precautions
Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred to in safety data sheets of the product.

Quality Control
Each lot of Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. It has been functionally tested in amplification assay.

Limitations
Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. This assay must not be used on the specimen directly. Viral DNA should be extracted from clinical specimen using appropriate nucleic acid extraction method. Presence of PCR inhibitors and other interferences may lead to false negative or invalid results. Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in failure to detect the presence of pathogen. As with any diagnostic test, results of the Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit need to be interpreted in consideration of all clinical and laboratory findings. Performance of the kit in monitoring treatment of CMV infection has not been evaluated.

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	No amplification in test and/or control wells	Degraded samples or poor-quality DNA template	Use freshly extracted, high-quality DNA. Check DNA concentration and purity (e.g., A260/280 ratio). Avoid repeated freeze-thaw cycles for stored DNA.
		Missing or incorrect addition of reagents	Verify all reagents were added in the correct volumes and order. Recheck reaction setup steps.
		Incorrect thermal cycling conditions	Cross-check the PCR cycling profile with the IFU.
		Expired or improperly stored reagents	Confirm the expiry date and proper storage. Avoid using reagents that have undergone multiple freeze-thaw cycles
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
2.	Variability between replicates	Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
		Air bubbles in reaction mix	Briefly centrifuge PCR tubes or plate before placing in the instrument to eliminate air bubbles.
		Uneven mixing of reagents	Ensure all reagents are fully thawed and mixed by vortexing before use. Spin down before pipetting.

		Edge effect (thermal variation across plate)	Avoid using outer wells in PCR plates if not temperature-uniform; use a plate seal and consistent plate layout.
3.	Amplification of pathogen target in negative control	Reagent contamination	Replace all critical reagents. Clean workspace, pipettes, and repeat analysis with fresh aliquots. Use filter tips and maintain a unidirectional workflow.
		Cross-contamination or aerosol contamination during reaction setup	Maintain strict unidirectional workflow. Set up reactions in a PCR hood or clean bench. Use aerosol-resistant filter tips for all pipetting steps. Avoid fast, forceful pipetting. Regularly clean work surfaces and equipment with DNA-decontaminating agents. Minimize opening of positive control tubes and avoid splashing.
		Template contamination in workspace or pipettes	Decontaminate work surfaces, pipettes, and equipment using DNA/RNA decontamination solutions. Perform regular cleaning.
		Improper sealing of PCR plate/tubes	Ensure plates/tubes are properly sealed to prevent cross-well contamination during thermal cycling. Use optical-grade seals if required.
4.	No signal with positive control (Quantitative standards) or partial target amplification	Degradation of the positive control material due to improper storage or repeated freeze-thaw cycles	Use a fresh aliquot of positive control. Ensure storage conditions follow IFU and avoid repeated freeze-thaw. Discard expired or compromised controls.
		Incorrect thermal cycling conditions	Cross-verify cycler settings with IFU. Ensure annealing/extension temperature and time are as specified.
		Improper mixing of positive control (PC) before use	Vortex and briefly spin down the positive control before adding it to the PCR mix. Mix well to ensure homogeneity.
5.	Early Ct value with non-sigmoidal amplification	Instrumental noise, background fluorescence, or non-specific amplification	Visually inspect amplification plots. Disregard flat or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very early Ct values (e.g., <6). These may not indicate true amplification. Repeat the assay if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance.
6.	Non-specific amplification of pathogen targets in samples and/or negative control	Improper threshold setting in the real-time PCR software	Visually inspect all amplification plots. Do not rely on Ct values alone. Manually adjust the threshold as recommended in the threshold setting table. Refer to the instrument-specific guidelines or user manual for proper threshold adjustment.

		Contamination of reagents or workspace	Use fresh aliquots of reagents. Prepare reactions in a contamination-free environment using separate areas for pre- and post-PCR steps. Use aerosol-resistant filter tips and routinely decontaminate surfaces and pipettes.
7.	No Internal Control (IC) amplification in target-positive sample	High load of target may suppress IC amplification	If valid Ct for target is observed, it is still considered a valid result
8.	High Ct values in positive samples (late amplification)	Low viral load or suboptimal sample	Results near the cut-off should be interpreted with caution. Repeat test using freshly extracted DNA. Confirm with orthogonal test if clinically significant.
9.	Inconclusive result (no amplification of target or IC)	Sample inhibition, extraction failure, or expired reagents	Repeat extraction or test with fresh sample. Check IC amplification to rule out PCR inhibition. Use validated extraction method and fresh reagents.
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
10.	Signal in only one replicate (of duplicate or triplicate reactions)	Pipetting error or borderline positivity	Repeat the test. If consistent upon retesting, interpret cautiously in context of clinical findings. Borderline cases (Ct of 38-39) may require repeat sampling or orthogonal testing (e.g., NGS). Use calibrated pipettes and proper technique.
11.	Standard Curve does not show expected PCR parameters such as efficiency and slope	Improper Mixing of standards	Thaw and mix the standard vials properly before setting the PCR run.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
12.	Viral DNA detected above the linear range of the assay	High viral load sample	Dilute the DNA elute and repeat the assay. Multiply the observed IU/mL value by dilution factor.

Safety Information

HiMedia's Hi-PCR® Cytomegalovirus (CMV) Probe PCR Kit for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

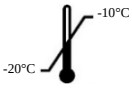
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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