

Quantiplus® CMV FAST RT PCR Kit (Real-Time Quantitative PCR Kit)



QTF-CMV-25 : 25 rxns
 QTF-CMV-50 : 50 rxns
 QTF-CMV-100 : 100 rxns



PI/QTF-CMV-02

Intended Use

Quantiplus® CMV FAST RT PCR Kit is a Real-Time PCR based in vitro diagnostic assay for quantitation of Cytomegalovirus in human plasma. The kit contains DNA Fast qPCR Mix with UDG/UNG, Primer Probe Mix (PPM), Standards (CMVFQS1-CMVFQS4), and Exogenous Internal Control (IC-B mix). This advanced formulation enables performance of fast PCR in shorter run time (≤ 60 min), and UDG/UNG helps in controlling PCR carryover contamination.

Background Information

Cytomegalovirus (CMV) belongs to the human herpes family. CMV spreads from person to person through body fluids, such as blood, saliva, urine, semen and breast milk. Infection with CMV remains for life but rarely causes problems in healthy people. CMV infection in immunocompromised patients may result in retinitis, pneumonia, intestinal or other organ specific disease. There is no cure, but there are medications that can help treat the symptoms.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QTF-CMV-25)	50 rxns (QTF-CMV-50)	100 rxns (QTF-CMV-100)
Blue	DNA Fast qPCR Mix with UDG/UNG (2X)	PCR Amplification Mix	1 x 325 μ L	1 x 650 μ L	2 x 650 μ L
Amber	CMV Fast PPM	Target specific Primer Probe Mix	1 x 50 μ L	1 x 100 μ L	2 x 100 μ L
Natural	IC-B Mix	Exogenous Internal Control-B mix	1 x 300 μ L	1 x 600 μ L	2 x 600 μ L
Pink	CMVFQS1	1 X 10 ⁴ IU/ μ L	1 x 100 μ L	1 x 100 μ L	2 x 100 μ L
Pink	CMVFQS2	1 X 10 ³ IU/ μ L	1 x 100 μ L	1 x 100 μ L	2 x 100 μ L
Pink	CMVFQS3	1 X 10 ² IU/ μ L	1 x 100 μ L	1 x 100 μ L	2 x 100 μ L
Pink	CMVFQS4	1 X 10 ¹ IU/ μ L	1 x 100 μ L	1 x 100 μ L	2 x 100 μ L
White	MBGPW	Purified Water	1 x 500 μ L	1 x 500 μ L	1 x 1.0 mL

Note: Please pay attention to the cap color coding and the tube contents.

MBGPW (Molecular Biology Grade Purified Water)

Storage and Transportation Conditions

The kits should be transported at temperature below -20 °C. The kit is stable until the expiry date printed on the package, if the storage temperature is within -20 ± 5 °C. More than 4X freezing and thawing cycles reduce the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Target Sequence	Specific region in CMV-UL65 of CMV genome
Specificity	100%
Sensitivity	0.13 IU/ μ L (65 IU/mL or 65 copies/mL)
Linear Range	1 x 10 ⁶ – 51.2 x 10 ⁻² IU/ μ L (5 x 10 ⁸ – 25.6 x 10 ¹ IU/mL or 5 x 10 ⁸ – 25.6 x 10 ¹ copies/mL)
Reporting Units	IU/ μ L (1 IU = 1 copy)
Validated Specimen	Plasma
External Quality Assessment	QCMD EQA Panels

Assay Procedure

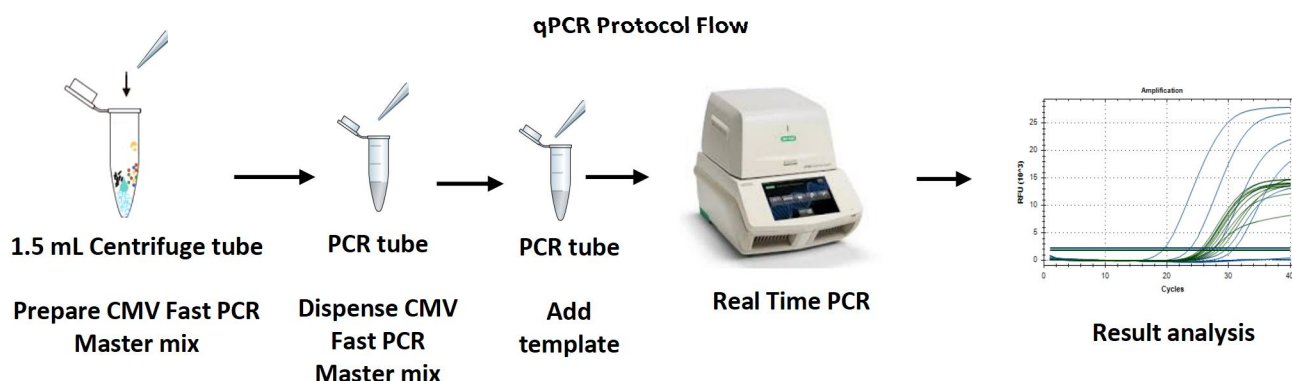
DNA Extraction

Quantiplus® CMV FAST RT PCR Kit has been validated using the following Viral DNA extraction kits:

Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
2.	QIAamp DNA Blood Mini Kit (Cat. No. 51104)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other Viral DNA extraction Kits.



Preparation of Reaction Master mix

Components	Volume per reaction (For 26 µL)
DNA Fast qPCR Mix with UDG/UNG (2X)	13.0
CMV Fast PPM	2.0
IC-B Mix (if not added at extraction step)	1.0
Extracted DNA/ CMVFQS1- CMVFQS4 / MBGPW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes, centrifuge briefly before proceeding to thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	1 min.
2 (PCR cycling)	45	95	10 sec.
		60*	10 sec.

***Plate read/Data acquisition in FAM and TEXAS RED channels in Bio-Rad TM CFX 96. For Thermo QS5 Real-Time PCR System, use FAM and ROX channels. or Rotor-Gene Q 5 plex, use Green and Orange channels.**

Sample analysis and Interpretation

The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below and also the slope of the standard curve (standards in FAM channel) is between -3.1 to -3.6, at least three standards should be included, and PCR efficiency is between 90% to 110% (0.9 to 1.1). Interpret the results of unknown samples as mentioned in Table 2

Table 1:

Control	FAM (CMV)	TEXAS RED (IC)
If Internal Control (IC-B Mix) is added during extraction		
Standards (CMVFQS1 to CMVFQS4)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Standards (CMVFQS1 to CMVFQS4)	√	√
Negative Control (NC)	-	√

Table 2:

S.No	FAM (CMV)	TEXAS RED (IC)	Interpretation	Fluorophore	Conclusion
1	√	√	CMV DNA detected within quantitation range	←	Proceed for further Analysis
2	√	-			
3	-	√	CMV DNA below quantitation limit		
4	-	-	Possible inhibition of PCR		Dilute the DNA sample (1:10) and repeat the Assay

Note: All the Target channels (FAM, Texas Red) to be analyzed individually

Viral load calculation (Conversion of IU/μL to IU/mL)

$$\text{IU/mL} = \frac{\text{Obtained IU/}\mu\text{L} \times \text{Elution Volume}(\mu\text{L})}{\text{Sample volume in mL}}$$

For calculating the result of diluted sample (1:10); multiply the observed IU/mL value by dilution factor, 10

$$\text{Result of 1:10 diluted sample (IU/mL)} = \text{Dilution Factor (10)} \times \frac{\text{Result (IU/}\mu\text{L)} \times \text{Elution Volume } (\mu\text{L})}{\text{Sample Volume (mL)}}$$

Reporting comments

Results in IU/mL	Comments
Target not detected	CMV DNA not detected in the given sample
<256	CMV DNA detected but below the lower limit of the linear range of the assay. The reproducibility of the positive result is not assured
256 to 5×10^8	CMV DNA detected within the linear range of the assay
$\geq 5 \times 10^8$	CMV DNA detected but above linear range of the assay, dilute the sample and repeat the assay for accurate result.

Validated Instruments

- Thermo Q55 Real-Time PCR System
- Bio-Rad™ CFX 96



HLSS Manufacturing Pvt Ltd
Plot No's M14, M15, M16, TSIC Medical device park
Sultanpur villiage, Ameenpur Mandal,
Sangareddy Dist, TS-502319