# RTA®
# CMV Real-Time PCR Kit Handbook

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**RUO**

**REF** 09063025 - 25tests
09063100 - 100 tests

Quantitative detection of Human Cytomegalovirus DNA

For research use only

## Kit Contents

| | CapColor | Reagents | 25 Tests | 100 Tests |
|---|---|---|---|---|
| 1 | BROWN | CMV Mix A | 300 µl | 1150 µl |
| 2 | YELLOW | CMV Mix B | 250 µl | 950 µl |
| 3 | BLUE | CMV Internal Control | 75 µl | 250 µl |
| 4 | RED | CMV Quantification Standart 1 ($10^7$ IU/ml) | 100 µl | 100 µl |
| 5 | RED | CMV Quantification Standart 2 ($10^6$ IU/ml) | 100 µl | 100 µl |
| 6 | RED | CMV Quantification Standart 3 ($10^5$ IU/ml) | 100 µl | 100 µl |
| 7 | RED | CMV Quantification Standart 4 ($10^4$ IU/ml | 100 µl | 100 µl |
| 8 | WHITE | PCR Grade Water | 100 µl | 100 µl |

## Storage

All reagents of RTA CMV Real-Time PCR Kit should be stored at -20°C. Storage at higher temperatures should be avoided (e.g. +4°C). Under these conditions, kit contents should be stable through the expiration date printed on the label. The reagents should not be freeze-thawed more than 2 times; otherwise the shelf life of the kit will reduce. During the working steps all reagents should be kept on ice.

## Intended Use

RTA CMV Real-Time PCR Kit is an in vitro nucleic acid amplification assay for quantification of Cytomegalovirus (CMV) DNA in human serum or plasma (EDTA) using RTA Viral DNA Isolation Kit or RTA Viral Nucleic Acid Isolation Kit and Stratagene Mx3000p/Mx3005p instrument or Rotor-Gene 3000/6000 or INCEPTRA Cycler 4840/9620/9640/9660/9680 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD Real-Time PCR Detection System for amplification, detection and analysis. RTA CMV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic CMV infection in conjunction with all relevant clinical and laboratory findings. RTA CMV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of CMV DNA or confirmation of the diagnosis of infection with CMV.

## Product Use Limitations

- All reagents of the kit is for in vitro diagnostic use only.
- RTA CMV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of CMV DNA or confirmation of the diagnosis of infection with CMV.
- This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Test with other sample types may result in inaccurate results.
- This kit has been validated for use with RTA Viral DNA Isolation Kit or RTA Viral Nucleic Acid Isolation Kit. Using other isolation kits may adversely affect the performance characteristics of the kit.
- This kit has been validated for use with Stratagene Mx3000p/Mx3005p instrument or INCEPTRA Cycler 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD Real-Time PCR Detection System. Using other instruments may adversely affect the performance characteristics of the kit.
- This kit has been optimized for use with specific PCR plastic consumables listed under Additional Materials Required section of the Handbook. Using other PCR plastic consumables may adversely affect the performance characteristics of the kit.
- Trustworthy results depends on proper sample collection, transport, storage and processing methods.
- It is intended for professional use by properly trained personnel.
- RTA CMV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic CMV infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings.
- The instructions in user manual should be followed strictly for optimum PCR results.
- The expired kits should not be used. Kit components from different lots should not be mixed.

## Product Description

RTA CMV Real-Time PCR assay is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' nucleolytic activity of the Thermus aquaticus (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line. Quantification of the amount of target in unknown samples is accomplished by measuring Ct and using the standard curve to determine starting copy number. RTA CMV real-time PCR assay utilizes external standards to gather quantitative results and includes an internal control, which controls for target isolation and amplification. The target region is situated in UL54 gene region of CMV genome and is 176-bases long. CMV DNA concentration is noted in International Units/ml (IU/ml).
If it is needed to convert to copies/ml, our conversion factor for RTA CMV Real-Time PCR Kit is 0.92 copies/IU. In other words, 1 IU/ml = 0.92 copies/ml

## Pathogen Information

Cytomegaloviruses (CMVs) belong to the betaherpesviruses subfamily of herpesviruses and are ubiquitous but highly species-specific viruses that infect many animals, including humans. Infection with CMV is common in all populations and rarely associated with symptomatic infection in normal hosts. In contrast, it is a major cause of multiorgan disease in immunocompromised patients. CMV is also a leading cause of congenital infection and a leading infectious cause of brain disease and hearing loss in children in the United States and western Europe. As with other herpesviruses, primary infection with CMV is followed by a persistent infection (1).
CMV is morphologically similar to other herpesviruses and is the largest member of the family. The virus consists of a 64-nm core enclosed by a 110-nm icosahedral capsid. The capsid is surrounded by a poorly defined amorphous tegument that itself is surrounded by a loosely applied, lipid-containing tegument (2). The genome of CMV consists of linear double-stranded deoxyribonucleic acid (DNA) molecule approx 240 kb. The genome of CMV is similar to that of herpes simplex virus in that it has long and short unique segments, both of which are bounded by homologous repetitive sequences. The CMV genome is approx 50% larger than herpes simplex virus and encodes for at least 35 structural proteins and an undefined number of nonstructural proteins (3). Although the replication of CMV is very similar to that described for herpes simplex virus, the replicative cycle is much slower than for herpes simplex (4).


**References**

1 Weller TH. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. N Engl J Med 1971;285:203–214.

2 Smith J, DeHavern E. Herpes simplex and human cytomegalovirus replication in WI-38 cells. I. Sequence of viral replication. J Virol 1978;12:919–930.

3. Sarov I, Abady I. The morphogenesis of human cytomegalovirus. Isolation and polypeptide characterization of cytomegalovirus and dense bodies. Virology 1975;66:464–473.

4. Honess RW, Roizman B. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J Virol 1974;14:8–19.


## Warnings and Precautions

- All clinical specimens and all resulting waste materials should be treated as potentially infec- tious; the samples should be prepared in Bio-safety Level 2 area.
- Before and after work all surfaces should be disinfected with a freshly prepared solution of 10% bleach or antiviral agents.
- Dispose of unused reagents, waste and specimens in accordance with country or local regu- lations.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wear protective disposable gloves, laboratory coats and eye-wear when handling clinical specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water.
- The procedures should preferably be performed in four separated areas (i.e. for RNA extrac- tion, PCR setup, sample addition, amplification) to aid in preventing contamination. All supp- lies for a particular procedure should be stored in the area where that procedure is performed and should not be moved between areas Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to an area and never worn outside of that area. The work should flow in one direction, beginning in the extraction area, moving to the PCR setup area in which PCR Master Mix is prepared, then moving to the third area in which samples, negative control and quantification standards are added, finally moving to amplification area in which real-time PCR equipment is run.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control; to prevent sample contamination, use new, sterile aerosol barrier or positive displacement Rnase/DNase-free pipette tips and sterile pipettes.
- Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
- Store the kit away from any source of contaminating RNA or RNA, especially amplified nucleic acid.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- A single type of CMV DNA assay should be used for monitoring a patient. If RTA CMV Real- Time PCR Kit substitutes another CMV DNA assay, both tests should be used in parallel for at least two subsequent samples.
- Do not use a kit after its expiration date.

## Performance Characteristics

**Analytical Sensitivity** Analytical sensitivity was analyzed by use of a dilution series of WHO standard, and the cutoff value of the kit was determined by probit analysis. A dilution series of a WHO International CMV standard was prepared to give the final concentrations of 640, 320, 160, 80, 40 and 20 IU/ml. Dilutions were extracted by RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029) according to RTA Viral Nucleic Acid Isolation Kit Handbook. Each dilution was tested in 24 replicates for each instrument. Lower limit was calculated by probit analysis done by PASW Statistics 18 program. The

95 % cutoff concentration of RTA CMV Real-Time PCR Kit is 116 IU/ml for STRATAGENE, 103 IU/ml for INCEPTRA Cycler, 93 IU/ml for Rotor-Gene, 60 IU/ml for BIO-RAD CFX96-IVD and 77 IU/ml for Applied Biosystems 7500.

| | INCEPTRA Cycler | STRATAGENE | BIO-RAD CFX96-IVD* | Rotor-Gene | ABI 7500 |
|---|---|---|---|---|---|
| RTA Viral Nucleic Acid Isolation Kit | 103 IU/ml | 116 IU/ml | 60 IU/ml* | 93 IU/ml* | 77 IU/ml |

*: Data taken from RTA Automated DNA/RNA Preparation and PCR Setup System

**Linear Range** To determine the upper limit, a dilution series of RTA CMV Reference Material ranging from 1 x $10^3$ IU/ml to 1 x $10^6$ IU/ml were prepared. High concentration samples (1 x $10^7$, 1 x $10^8$ and 1 x $10^9$ IU/ml) were prepared by using calibrated plasmid DNA bearing CMV external standard. Within this range, the relationship between log of target DNA and Ct values is linear. Linear regression analyses comparing the Ct values-versus- log of target DNA were as follows:

For STRATAGENE, Ct value = -3.490(log of target DNA) + 45.50; with a correlation coefficient (R2) of 0.988.
For INCEPTRA Cycler, Ct value = -3.39(log of target DNA) + 47.32; with a correlation coefficient (R2) of 0.980.
For BIO-RAD CFX96, Ct value = -3.71(log of target DNA) + 49.19; with a correlation coefficient (R2) of 0.998.
For Rotor-Gene, Ct value = -3.21(log of target DNA) + 41.08; with a correlation coefficient (R2) of 0.998.
For ABI 7500, Ct value = -3.54(log of target DNA) + 43.99; with a correlation coefficient (R2) of 0.999.

Upper limit is at least 1 x $10^9$ IU/ml for STRATAGENE, INCEPTRA Cycler, Rotor-Gene and ABI 7500.

Lower limit was calculated by probit analysis done by PASW Statistics 18 program according to the quantification results of CMV Analytical Sensitivity Studies. 95 % lower confidence limit is 135 IU/ml for STRATAGENE, 103 IU/ml for INCEPTRA Cycler, 58,5 IU/ml for BIO-RAD CFX96- IVD, 102 IU/ml for Rotor-Gene and 86,5 IU/ml for ABI 7500. Dynamic ranges of RTA CMV Real-Time PCR Kit:

**For STRATAGENE** **135 - 1 x $10^9$ IU/ml**
**For INCEPTRA Cycler** **103 - 1 x $10^9$ IU/ml**
**For Rotor-Gene** **102 - 1 x $10^9$ IU/ml**
**For ABI 7500** **86,5 - 1 x $10^9$ IU/ml**
**For BIO-RAD CFX96-IVD** **58,5- 1 x $10^9$ IU/ml***

*: Data taken from RTA Automated DNA/RNA Preparation and PCR Setup System

**Precision** For each experiment, 24 replicates of $10^4$ IU/ml WHO International Standard for CMV DNA assays were used. The results on basis of Ct values are shown in the following table:

| Descriptive Statistics | | | | | |
|---|---|---|---|---|---|
| | N | Mean | Std.Deviation | Variance | Coefficient of variation (%) |
| Intra_assay | 24 | 28.76 | 0.39 | 0.153 | 1.36 |
| Inter_assay | 24 | 28.79 | 0.38 | 0.149 | 1.34 |
| Inter_batch | 24 | 28.76 | 0.59 | 0.354 | 2.07 |
| INCEPTRA_Cycler_9660 | 24 | 28.59 | 0.39 | 0.158 | 1.39 |
| BIORAD | 24 | 29.31 | 0.23 | 0.056 | 0.81 |
| Rotor-Gene | 24 | 28.73 | 0.13 | 0.018 | 0.47 |
| ABI7500 | 24 | 29.22 | 0.23 | 0.056 | 0.81 |
| TOTAL | 168 | 28.88 | 0.44 | 0.194 | 1.52 |

**Diagnostic Specificity** CMV negative clinical specimens were analyzed to determine the diag- nostic specificity of RTA CMV Real-Time PCR Kit. 63 CMV DNA negative clinical serum speci- mens and 53 CMV DNA negative clinical EDTA plasma specimens were used. Viral DNA was extracted from CMV negative clinical specimens by RTA Viral DNA Isolation Kit according to RTA Viral DNA Isolation Kit Handbook.
None of the 116 CMV negative clinical specimens gave positive test result for CMV DNA. Diag- nostic specificity of RTA CMV Real-Time PCR Kit is 100 %. All of the Internal Controls of tests gave positive result.

**Cross Reactivity** To examine the specificity of an assay, cross-reactivity studies should be per- formed for potential cross-reactive markers. In this study, the specificity of the assay was evalu- ated by testing 8 reference organisms and 10 clinical specimens which were positive. RTA CMV Real-Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the following table:

| *Organism* | Source | Test Result |
|---|---|---|
| Hepatitis B virus (HBV) | NIBSC (Cat. No: 97/750) | Negative |
| Human Herpes Simplex virus type 1 (HSV-1) | NIBSC (Cat. No: 08/224) | Negative |
| Human Herpes Simplex virus type 2 (HSV-2) | NIBSC (Cat. No: 08/226) | Negative |
| Epstein-Barr Virus (EBV) | NIBSC (Cat. No: 08/316) | Negative |
| Hepatitis C virus (HCV) | NIBSC (Cat. No: 06/100) | Negative |
| Human Immunodeficiency Virus 1 (HIV-1) | NIBSC (Cat. No: 97/650) | Negative |
| Mycobacterium tuberculosis | ATCC (25177) | Negative |
| Human Immunodeficiency Virus 2 (HIV-2) | NIBSC (Cat. No: 08/150) | Negative |
| Hepatitis B virus (HBV) | Clinical specimens | Negative |
| Hepatitis B virus (HBV) | Clinical specimens | Negative |
| Hepatitis C virus (HCV) | Clinical specimens | Negative |
| Hepatitis C virus (HCV) | Clinical specimens | Negative |
| Human Immunodeficiency Virus 1 (HIV-1) | Clinical specimens | Negative |
| Human Immunodeficiency Virus 1 (HIV-1) | Clinical specimens | Negative |
| Parvovirus B19 | Clinical specimens | Negative |
| Epstein-Barr Virus (EBV) | Clinical specimens | Negative |
| Hepatitis D virus (HDV) | Clinical specimens | Negative |
| Human papillomavirus type 6 (HPV 6) | Clinical specimens | Negative |

**Cross-Contamination** In this study, cross-contamination between samples was evaluated. To do this, five different runs were performed. In every run, 4 high positive CMV sample and 4 CMV negative samples were used. Then, the kit was evaluated accordingly whether or not any cross-contamination was observed.No cross-contamination was observed during the whole process, and none of the human serum samples exhibited evidence of containing PCR inhibitors as indicated by the amplification of internal control.

**Whole System Failure**

56 CMV negative clinical serum specimens and 52 CMV DNA negative clinical EDTA plasma specimens were spiked with 1st WHO International Standard for Human Cytomegalovirus (NIBSC code: 09/162) to give a final concentrations of 345 IU/ml in the elution volume which is 3 times the 95% positive cutoff value determined by analytical sensitivity study. Spikes were extracted by RTA Viral Nucleic Acid Isolation Kit according to RTA Viral Nucleic Acid Isolation Kit Handbook. Whole system failure rate of RTA CMV Real-Time PCR Kit is ≤1 %.

**Clinical Comparative Study**

Total 157 clinical samples were tested. According to the results, the data gathered by RTA CMV Real-Time PCR Kit is compatible with the results of other CE- marked devices. Log concentrations of all of 157 positive clinical samples are between ±1 log concentrations of the result of comparative device.

## Additional Materials Required

- RTA Viral DNA Isolation Kit (Cat No: 09006; RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey),
- Real-Time PCR system,
- Disposable powder-free gloves
- Micropipettes (0.5 µl – 1000 µl),
- Sterile micropipette tips with filters,
- Microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for 2.0 ml tubes and for PCR strip tubes,
- PCR Workstation,

Real-Time PCR reaction tubes/plates/capillaries
For BIO-RAD CFX96-IVD:

- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates with Bar Codes (BIO-RAD, Cat#: HSP-9955)
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001),
- Individual PCR Tubes, Low Tube Strips, 8-tubes strip, 0.2 ml Low Profile, White (BIO-RAD, Cat#: TLS0851)
- Flat Cap Strips, Optically Clear, 8-cap strip, 0.2 ml (BIO-RAD, Cat#: TCS0803)

For STRATAGENE MX 3000p/MX 3005p:

- Optical tube strips (8x Strip) (Agilent Technologies, Cat#: 401428),
- Strip caps for PCR and qPCR applications (Agilent Technologies, Cat#: 401425)

For Rotor-Gene 3000/6000:

- Strip Tubes and Caps, 0.1 ml (Qiagen, Cat#: 981103)

For INCEPTRA Cyclers:

- 96x0,2 ml plate (Bioplastics, Cat#: B70501). EU flat cap plate (Bioplastics, Cat#: B57601),EU 0.1ml 8-tube strip attached Optical wide area cap (Bioplastics, Cat#: K72810B)

For Applied Biosystems 7500:

- MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher, Cat#: 4306737)
- MicroAmp® Optical Adhesive Film (Thermo Fisher, Cat#: 4311971)
- MicroAmp® Optical 8-Tube Strip, 0.2 mL (Thermo Fisher, Cat#: 4316567)
- MicroAmp® Optical 8-Cap Strips (Thermo Fisher, Cat#: 4323032)

## Sample Preparation

This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant.
Aseptic techniques must be employed during collection to prevent the introduction of micro-organisms into the patient's anatomical space, and to prevent the sample from being contaminated during the process of collection.
All samples should be regarded as potentially infectious and standard precautions guidelines should be followed by all healthcare workers during sample collection and handling.
Samples must be collected into appropriate containers before despatch to the laboratory.
Be careful to check for cracks in the containers and to ensure that the lids of containers are properly tightened to prevent leakage of samples during handling and transportation. This can pose infection hazards to transport and laboratory staff.
Ensure that the outer surfaces of the containers are not contaminated by the patients' samples.
Store whole blood at room temperature for no longer than 4 hours. Centrifuge blood within 4 hours of collection. Transfer serum or plasma to a screw cap cryovial tube.
Transportation of whole blood, serum or plasma must conform to country or local regulations for the transport of etiologic agents.
Serum or plasma samples may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder for long-term storage.
Avoid multiple freeze/thaw cycles of specimens.

## Protocol

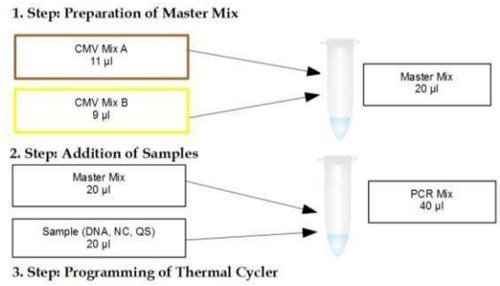
**Viral DNA Isolation**
RTA Viral DNA Isolation Kit (Cat No: 09006; RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey) should be used for viral DNA extraction from clinical samples. Please follow the manufacturer's instructions as stated in the kit manual

**Internal Control**
During DNA isolation, addition of the supplied internal control (IC) is necessary. IC allows the user to monitor RNA extraction step as well as to determine any PCR inhibition. For each sample, add 2.5 µl IC together with Solution RL of the isolation kit for a 50 µl elution. Depending on your final elution volume, the volume of IC to be added can be calculated (0.05 µl IC/1 µl Elution Buffer). There will be no amplification of internal control in the tests where high positive CMV samples are amplified because there is a competition between internal control template and CMV DNA template for using PCR primers and other components. The Ct value of internal control of a negative sample should be equal to 33 ± 5, otherwise, it denotes a problem during purification.

**Quantification Standards** For generating a standard curve to obtain accurate quantification data on the Real-Time system, four quantification standards should be used. For each standard the corresponding concentration should be defined properly to the Real-Time PCR system be- fore each run and the standard curve will be generated accordingly at the end of the reaction. Work with CMV Quantification Standards after preparation of clinical samples and negative cont- rol in a separate area. Caps of the tubes or capillaries of Clinical Samples SHOULD be closed in that area.

**PCR Protocol**

1. Thaw all components, except CMV Enzyme Mix, at room temperature. Thaw CMV Reaction Mix at 37°C for 5 min if there is a precipitate. Put CMV Enzyme Mix on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.
2. The final volume of Master Mix is calculated by multiplying single reaction volumes of Reaction Mix and Enzyme Mix by the total sample size. The number of negative controls, quantification standards and the clinical samples should be included when calculating total sample size. Aga- inst possible pipetting errors, addition of an extra sample to the total sample size is recommen- ded. PCR Grade Water should be used as the negative control.
3. To prepare master mix, add 11 µl of CMV Mix A (brown tube) and 9 µl of CMV Mix B (yellow cap) for each sample to the master mix tube. Vortex the tube and spin down briefly in a micro- centrifuge. Add 20 µl of Master Mix into Real-Time PCR reaction tubes for each sample. Add 20 µl DNA of each sample, negative control and quantification standards into the tubes. Spin down briefly.
4. Perform the following protocol for all PCR cyclers: 95°C for 10 min, 1 cycle; 95°C for 30 sec, 60°C for 60 sec, 72°C for 30 sec, 45 cycles.
5. Fluorescence is measured at 72°C; FAM and HEX channels should be chosen (See the schema in the next page).
6. Refer to the Operator's Manual of the related instruments to program and analyze the results.




| Program Name | Cycles | Program for All Cycler |
|---|---|---|
| Hot Start | 1 | 95ºC, 10 min |
| Amplification* | 45 | 95ºC, 30 sec |
| | | 60ºC, 60 sec |
| | | 72ºC, 30 sec |

*** Fluorescence is measured at 72°C; FAM and HEX channels should be chosen**

## Data Analysis

To be able to evaluate the experiment, PCR efficiency of the Standard Curve must be between 90%-110% and R2 value must be more than 0.98. Otherwise, the experiment should be repeated.
During analysis on STRATAGENE software, adjust threshold fluorescence value manually by entering 1000 for FAM and 500 for HEX.
During analysis on BIO-RAD CFX96-IVD software, adjust threshold fluorescence value manually by entering 500 for FAM and 500 for HEX.
During analysis on INCEPTRA Cycler software, the threshold should be set empirically. At the beginning, the threshold can be set at 50 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Rotor-Gene software, the threshold should be set empirically. At the beginning, the threshold can be set at 0,04 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Applied Biosystems 7500 software, the threshold should be set empirically. At the beginning, the threshold can be set at 10,000 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
Concentration of each positive sample will be calculated by the software according to the stan- dard curve as International Unit per milliliter (IU/ml) . If it is needed to convert the quantitative results from IU/ml to copies/ml, the conversion factor for RTA CMV Real-Time PCR Kit is 0.92 copies/IU. In other words, 1 IU/ml = 0.92 copies/ml.
Due to different starting sample volumes and elution volumes during viral DNA isolation, the following formula SHOULD be used to calculate the concentration of the original clinical sample:

$$\text{Concentration of the Original Sample (IU/ml)} = \frac{\text{Concentration from Software (IU/ml)} \times \text{Elution Volume (µl)}}{\text{Original Sample Volume (µl)}}$$

The interpretation on the calculated results can be done as follows:

| Signal detected in FAM channel | Signal detected in HEX channel | Calculated concentration of the original clinical sample | Conclusion |
|---|---|---|---|
| + | + | <60 IU/ml for BIO-RAD<br><116 IU/ml for STRATAGENE<br><103 IU/ml for INCEPTRA<br><102 IU/ml for Rotor-Gene<br><86,5 IU/ml for ABI 7500 | The result is valid.<br>Quantitation is not possible since the quantitative result is below the analytical sensitivity value of the assay. Reproducibility of the positive result is not guaranteed. |
| + | +/- | ≥60 IU/ml for BIO-RAD<br>≥116 IU/ml for STRATAGENE<br>≥103 IU/ml for INCEPTRA<br>≥102 IU/ml for Rotor-Gene<br>≥86,5 IU/ml for ABI 7500<br>and<br>≤1 x $10^9$ IU/ml | The result is valid.<br>CMV DNA is detected at the concentration calculated by the software since the quantitative result is within the linear range of the assay. |
| + | +/- | >1 x $10^9$ IU/ml | The result is valid. CMV DNA is detected at a concentration >1 x $10^9$ IU/ml . Quantitation is notpossible since the quantitative result is above the linear range of the assay. |
| - | + | N/A | The result is valid.<br>Target (CMV DNA) is not detected. |
| - | - | N/A | The result is invalid.<br>No diagnostic interpretation can be done, |

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