# SimReal™ CMV Detection & Quantitation Kit

Cat. No: SBL11-197

**USER MANUAL**

## INTRODUCTION

Cytomegalovirus (CMV) is a member of the Herpesviruses group (designated as HHV-5). CMV belongs to the β-herpesvirus subfamily of Herpesviridae. Cause mononucleosis, and pneumonia in normal individuals but may leads to life-threatening infections in immunosuppressed patients (e.g. patients with HIV, organ transplant recipients, or infants).

All herpes viruses share a characteristic ability to remain latent within the body over long periods of time. CMV can be found in numerous body fluids including urine, saliva, breast milk, blood, tears, and semen. CMV infection is the most significant viral cause of birth defects.

## KIT CONTENTS

| Components | Labels | Cap Color | Volume |
|---|---|---|---|
| | | | 25 Tests |
| 2x Reaction Mix | Reaction Mix | Black | 250 µl |
| Primer and Probes mix | Oligomix | White | 50 µl |
| Standard 1 ($10^2$ copy/µl) | STD 1 | Green | 20 µl |
| Standard 2 ($10^3$ copy/µl) | STD 2 | Yellow | 20 µl |
| Standard 3 ($10^4$ copy/µl) | STD 3 | Red | 20 µl |
| Standard 4 ($10^5$ copy/µl) | STD 4 | Violet | 20 µl |
| Standard 5 ($10^6$ copy/µl) | STD 5 | Blue | 20 µl |

## TEST PRINCIPLE

The SimReal™ CMV Kit is an in-vitro diagnostic kit designed for specific detection and quantitation of CMV infection on the basis of in-vitro DNA amplification using Real-time PCR technology.

The viral genome detection is based on amplification and detection of CMV conserved sequence using a corresponding labeled probe. The probe targeting viral sequence is labeled with FAM fluorochrome. Furthermore, viral genome quantitation can be performed using absolute quantitation method.

## PROTOCOL

### *a) Preparation of the reaction mix*

For each experiment, prepare a master mix of an appropriate volume for negative control, standard samples and (n+1) test samples. The reagents of the mix have to mix with this ratio:

| Component Labels | Volume/reaction |
|---|---|
| Reaction Mix | 10 µl |
| Oligomix | 2 µl |

Aliquot 18µl of Master Mix in each tube and add 2µl of extracted DNA or control DNA into individual tubes, spin tubes shortly and place them in your Real-time PCR device.

### *b) Real time PCR cycler programming*

Set device as the following profile:

| Step | Temp | Time | Data collection | Cycle |
|---|---|---|---|---|
| Initial activation | 95˚C | 15 min | | 1X |
| Denaturation | 95˚C | 30 s | * | 40X |
| Annealing | 58˚C | 40 s | | |
| Extension | 72˚C | 20 s | | |

**The acquisition must be performed in FAM.*

**Notice**: appropriate detection or quantitation reaction template files can be found in www.sim-biolab.com

Standard samples are necessary for tittering of viral load by standard curve quantitation method. At least three standards should be used, respectively, in order to provide standard curve, for example, $10^2$, $10^3$, $10^4$, $10^5$ and $10^6$**.**

You can use one of standards as unknown sample to evaluate reaction accuracy.

## DATA ANALYSIS

The fluorescence in each channel indicates the hybridization of the probe

**Channel 1 for FAM= CMV specific probe**

**Channel 2 for HEX= internal control probe**

If a sample shows fluorescence in the green channel, the sample is positive for virus genome **.**

For estimating viral titer, absolute quantitation method should be used **.**

- You can download absolute quantitation program from www.sim-biolab.com

Standard curve design and calculate the CMV DNA in each sample, automatically. These data are using for the CMV DNA concentration estimation in copies/ml according to below equation:

$$\frac{\textbf{Sample concentration } \left(\frac{\textbf{copy}}{\textbf{ul}}\right) \times \textbf{Elution volume (ul)}}{\textbf{Isolation volume (ml)}}] = \textbf{CMV copy/ml}$$

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