RUO

For Professional Use Only

Application note

Detection of human cytomegalovirus (CMV) using
eSens EBV/HHV6 QT PCR kit, REF ES3240A
for Research Use Only. Not for use in diagnostic procedures.

PURPOSE OF THIS APPLICATION NOTE

The purpose of this application note is to provide an **extended guidance** for utilizing the eSens EBV/HHV6 QT PCR kit. This document aims to assist laboratory professionals and researchers in accurately detecting the DNA of **not only** *Epstein-Barr virus* (*EBV*) DNA and *Human Herpes virus* type 6 (*HHV6*) in various clinical samples, but **it also introduces the supplementary feature of the kit that enables the detection and quantification of human cytomegalovirus (CMV) DNA for research use only (RUO)**, despite it not being CE IVD validated for this pathogen.

This application note provides specific supplementary data highligted by red color to facilitate the use of this feature.

PRINCIPLE OF PCR DETECTION

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5
DNA-target	IC Glob DNA	*EBV* DNA	*CMV* DNA	*HHV6* DNA
Target gene	β-globin gene	LMP-gene	exon 4 of MIE (major immediate early) gene	DNA polymerase catalytic subunit

PROTOCOL

Amplification

Fluorescent signal is detected in the channels for the FAM, JOE, ROX and Cy5 fluorophores.

DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

- The signal of the ***CMV* DNA** amplification product is detected in the channel for the **ROX** fluorophore

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- ***CMV* DNA is detected** if the *Ct* value determined in the results grid in the channel for the **ROX** fluorophore does not exceed the boundary *Ct* value specified in the *Technical Sheet*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***EBV* DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line), ***CMV* DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the ROX fluorophore (the fluorescence curve does not cross the threshold line) and ***HHV6* DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the Cy5 fluorophore (the fluorescence curve does not cross the threshold line). Whereas for qualitative analysis the *Ct* value in the results grid in the channel for the FAM fluorophore should not exceed the *Ct* value specified in the *Technical Sheet,* and for quantitative analysis, the quantity of IC Glob DNA should be more than 2000 copies/reaction for whole blood, white blood cells, viscera biopsy material.
- The result of analysis is **invalid** if the *Ct* value is not determined (absent) in the results grid or greater than the boundary *Ct* value in the channels for the JOE, ROX or Cy5 fluorophores. Whereas the *Ct* value in the results grid in the channel for the FAM fluorophore is greater than the *Ct* value specified in the *Technical Sheet* (for qualitative analysis) or the quantity of IC Glob DNA is less than 2000 copies/reaction for whole blood, white blood cells, viscera biopsy material (for quantitative analysis). In such case the PCR analysis should be repeated for required sample.
- The result is **equivocal** for the clinical samples with the *Ct* value determined in the channels for the ROX, JOE or Cy5 fluorophores greater than the boundary *Ct* value specified in the *Technical Sheet*. In that case, it is necessary to conduct additional analysis for that DNA sample with two repeats. If the repeated positive *Ct* value is obtained, the result is considered positive. If the positive *Ct* value can't be reproduced in two repeats, the result is considered **equivocal.**

Table 4

Results for controls in qualitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
C–	DNA extraction, PCR	Absent	Absent	Absent	Absent
PCE	DNA extraction, PCR	<boundary value	<boundary value	<boundary value	<boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+ (for qualitative analysis)	PCR	<boundary value	<boundary value	<boundary value	<boundary value

Table 5

Results for controls in quantitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
C–	DNA extraction, PCR	Absent	Absent	Absent	Absent
PCE	DNA extraction, PCR	<boundary value	concentration value falls in the range specified in the *Technical Sheet*	concentration value falls in the range specified in the *Technical Sheet*	concentration value falls in the range specified in the *Technical Sheet*
NCA	PCR	Absent	Absent	Absent	Absent
KSG1, KSG2	PCR	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined

For quantitative analysis, if total DNA is extracted from human whole blood, white blood cells, and viscera biopsy material, the concentration in log of DNA copies per standard cell quantity (10^5) in control and test samples is calculated according to the following formula:

For *CMV*:

$$\lg\left\{\frac{\text{number of } CMV \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \text{ x 2 x } 10^5\right\} = \lg\{CMV \text{ DNA copies}/10^5 \text{ cells}\}$$

If total DNA is extracted from cerebrospinal fluid (liquor), the concentration of DNA per ml of clinical sample (CS $_{DNA}$) is calculated according to the following formula:

CS $_{DNA}$ = number of DNA copies CMV, *EBV*, *HHV*6 in PCR sample x 100 (copies/ml)

SPECIFICATIONS

Sensitivity

Clinical material	Nucleic acid extraction kit	Analytical sensitivity
Cerebrospinal fluid (liquor)	RIBO-prep ePure Viral Nucleic acid extraction kit	400 copies/ml
Whole blood, white blood cells, viscera biopsy material	RIBO-prep ePure Viral Nucleic acid extraction kit	5 DNA copies per 10^5 cells