CE 1023

IVD For *in Vitro* Diagnostic Use

For Professional Use Only

CMV Real-TM

Handbook

Real Time PCR kit for the qualitative detection of *Cytomegalovirus* (CMV)

REF V7-100FRT

Σ 100

NAME

CMV Real-TM

INTENDED USE

CMV Real-TM PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of human cytomegalovirus (*CMV*) DNA in the clinical materials (urogenital swabs, urine samples, saliva, whole human blood) by using real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

PRINCIPLE OF ASSAY

CMV DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **CMV Real-TM** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

MATERIALS PROVIDED

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FRT *CMV*	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-) *	colorless clear liquid	1.2	1 tube
Internal Control-FL **	colorless clear liquid	1.0	1 tube

Contains reagents for 110 tests.

* *must be used in the isolation procedure as Negative Control of Extraction.*

** *add 10 µl of Internal Control during the DNA isolation directly to the sample/lysis mixture (see DNA-Sorb-A REF K-1-1/B/100 protocol).*

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers.
- Disposable polypropylene 1,5/2,0 ml tubes.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes.
- PCR Workstation.
- Real Time Thermal cycler.
- Disposable polypropylene microtubes for PCR.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

IVD ***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
9. Material Safety Data Sheets (MSDS) are available on request.
10. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
11. PCR reactions are sensitive to contamination. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practice.
12. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the PCR and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

⚠ *Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.*

⚠ *Sampling of biological materials for PCR-analysis, transportation, and storage are described in details in the handbook of the manufacturer. It is recommended that this handbook is read before beginning of the work.*

STORAGE INSTRUCTIONS

The components of the **CMV Real-TM** PCR kit must be stored at 2–8 ºC excepting **Polymerase (TaqF)** and **PCR-mix-2-FRT** that must be stored at -16°C or below.

The kit can be shipped at 2-8°C for no longer than 5 days but should be stored at 2-8°C and -16°C or below immediately on receipt.

STABILITY

CMV Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

The shelf life of reagents before and after the first use is the same, unless otherwise stated.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

CMV Real-TM can analyze DNA extracted from:

- *Whole blood* should be collected to a tube with 6% EDTA solution at a ratio 20:1 (20 portions of blood per 1 portion of EDTA) after overnight fasting.

   Do not freeze the whole blood samples!

- *cervical and urethral swabs*:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 mL of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.
- *urine sediment*: collect 10-20 ml of first-catch urine in a sterile container. Centrifuge for 30 min at 3000 x g, carefully discard the supernatant and leave about 200 µl of solution. Resuspend the sediment. Use the suspension for the DNA extraction.

Specimens can be stored at +2-8°C for no longer than 48 hours, or frozen at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇨ **DNA-Sorb-B** (Sacace, REF K-1-1/B/100);
- ⇨ **DNA/RNA-Prep** (Sacace, REF K-2-9);
- ⇨ **DNA-Sorb-A** (Sacace, REF K-1-1/A/100);

Please carry out DNA extraction according to the manufacture’s instruction.

Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.

PROTOCOL (Reaction volume 25 µl):

The total reaction volume is **25 µl**, volume of DNA sample - **10 µl**.

Unfreeze PCR-mix-2-FRT before mixing.

1. Prepare the required number of the tubes for amplification of DNA from test and control samples.
2. Prepare in a new sterile tube the **Reaction Mix.** For each sample mix **10*(N+1) µl** of **PCR-mix-1-FRT *CMV*, 5.0*(N+1) µl** of **PCR-mix-2-FRT** and **0.5*(N+1) µl** of **Polymerase (TaqF).** Mix the content of the tube by vortexing and then centrifuge shortly.
3. Add **15 µl** of **Reaction Mix** and **10 µl** of **extracted DNA** sample to appropriate tube. Mix by pipetting.
4. Carry out the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **Positive Control complex** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl** of **sample, isolated from Negative Control** to the tube labeled C- (Negative Control of Extraction).

Amplification

1. Create a temperature profile on your instrument as follows:

Step	Rotor type instruments[1]			Plate or modular type instruments[2]		
	Temperature, °C	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
Hold	95	15 min	1	95	15 min	1
Cycling	95	5 sec	5	95	5 sec	5
	60	20 sec		60	20 sec	
	72	15 sec		72	15 sec	
Cycling 2	95	5 sec	40	95	5 sec	40
	60	**20 sec fluorescence detection**		60	**30 sec fluorescence detection**	
	72	15 sec		72	15 sec	

[1] *For example, Rotor-Gene™ 3000 / Rotor-Gene™ 6000 / Rotor-Gene™ Q (Qiagen) or equivalent*
[2] *For example, , iCycler iQ™,/ iQ5™(BioRad), Mx3000P™ / Mx3000™ (Agilent) or equivalent.*

Cytomegalovirus is detected on the FAM (Green) channel, *IC DNA* on the JOE(Yellow)/HEX/Cy3 channel.

INSTRUMENT SETTINGS

Rotor-type instruments (for example RotorGene 3000/6000/Q)

Channel	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	*0.1*	*5 %*	*On*
JOE/Yellow	*0.1*	*5 %*	*On*

Plate- or modular type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

– ***CMV* DNA** amplification product is detected in the **FAM/Green** channel;

– **Internal Control** amplification product is detected in the **JOE/Yellow/HEX** channel.

RESULTS INTERPRETATION

The results are interpreted by the software of the instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

Principle of interpretation:

- *CMV* DNA is **detected** in a sample if its Ct value is present in the FAM channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.
- *CMV* DNA is **not detected** in a sample if its Ct value is absent in the FAM channel (fluorescence curve does not cross the threshold line) and the Ct value in the JOE channel is less than 33.
- The result is **invalid** if the Ct value of a sample in the FAM channel is absent while the Ct value in the JOE channel is either absent or greater than 33. It is necessary to repeat the PCR analysis of such samples.

Results for controls

Control	Stage for control	Ct value on channel		Interpretation
		FAM	JOE	
C–	DNA extraction	Neg	Pos (< 33)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< 33)	Pos (< 33)	OK

PERFORMANCE CHARACTERISTICS

Sensitivity

Analytical Sensitivity of **CMV Real-TM** PCR kit is the following:

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs or with Mucolytic	DNA-sorb-A	10^3
Urine (pretreatment is required)	–	DNA-sorb-A	$2x10^3$

***** *Genome equivalents (GE) of the pathogen agent per 1 ml of the sample placed in the transport medium*

Specificity

The analytical specificity of **CMV Real-TM** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: *Gardnerella vaginalis; Lactobacillus spp.; Escherichia coli; Staphylococcus spp.; Streptococcus spp.; Candida albicans; HSV types 1 and 2; Mycoplasma hominis; Ureaplasma urealyticum; Ureaplasma parvum; Mycoplasma genitalium; Neisseria flava; Neisseria subflava; Neisseria sicca; Neisseria mucosa; Neisseria gonorrhoeae; Trichomonas vaginalis; Treponema pallidum; Toxoplasma gondii; HPV.*

The clinical specificity of **CMV Real-TM** kit was confirmed in laboratory clinical trials.

Target region

Channel for fluorophore	FAM	JOE
DNA-target	CMV	Internal Control (IC)
Target gene	Pol gene	genetically engineered construction

QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

TROUBLESHOOTING

1. Weak or no signal of the IC (Joe/Hex/Cy3 channel) for the Negative Control of extraction.
    - The PCR was inhibited.
      ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
      ⇒ Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
    - The reagents storage conditions didn't comply with the instructions.
      ⇒ Check the storage conditions
    - Improper DNA extraction
      ⇒ Repeat analysis starting from the DNA extraction stage
    - The PCR conditions didn't comply with the instructions.
      ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
    - The IC was not added to the sample during the pipetting of reagents.
      ⇒ Make attention during the DNA extraction procedure.
2. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
      ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
3. Fam signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All sample results are invalid.
      ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
      ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
      ⇒ Repeat the DNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (DNA-buffer).
    - Contamination during PCR preparation procedure. All sample results are invalid.
    ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
    ⇒ Pipette the Positive control at last.
    ⇒ Repeat the PCR preparation with the new set of reagents.

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KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

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