Instructions For Use

LightMix® Modular human *Cytomegalovirus* (CMV)

500

Cat.-No. 50-0130-96

Kit with reagents for 96 PCR reactions 20 µl for quantification of CMV [lyophilized]

1. Content Storage and Expiry

1 Vial orange cap 96 reactions CMV (lyophilized)
1 Vial black cap Positive Control (≈ Cp 30), lyophilized
1 Standard row with 10 to 10^6 target equivalents per rxn

Storage at Arrival:

Store cooled or at ambient temperature
Do not freeze the lyophilized reagents.

- Kits are stable for one year after production (store 4°C to 25°C in the dark). See lot-specific expiry date.
- Reconstituted reagents are stable for two weeks if stored protected from light and cooled (2°C to 8°C).
- Dissolved reagent can be stored long-term if frozen (-15°C to -25°C). Avoid multiple freeze-thaw cycles.
- Reconstituted positive controls must be stored frozen. Minimize multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex DNA Master Cat.-No. 07 339 585 001

3. Introduction

Human Cytomegalovirus (hCMV), also known as Human Herpesvirus 5 (HHV-5), is a double stranded DNA virus and member of the herpes virus family. Primary infections of immunocompetent hosts are normally without symptoms; some individuals develop fever, and a very small percentage suffers from a mononucleosis syndrome or a mild hepatitis. About 50-80% of the population is positive for hCMV. Infections of unborn and young children as well as of immuno compromised individuals may cause severe symptoms and can be life threatening.

CMV will be tested in case of signs of mononucleosis and absence of EBV or symptoms of hepatitis and negative results for HAV, HBV or HCV. Diagnosis of hCMV infections is based on serology, virus culture or PCR.

4. Description

Two 151 (US17 gene) and 61 bp (F fragment gene) long fragments are amplified with specific primers (dual target PR) and detected with Cyan500 labeled hydrolysis probes.

5. Specification

This assay detects 10 genome equivalent copies or less per reaction (plasmid DNA dilution).

6. Sample Material and Extraction

Typical samples are peripheral blood samples for hCMV diagnosis.
For extraction protocols see Roche MagNA Pure or Roche manual kit instructions.

7. Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

This product is not hazardous, toxic, or IATA-restricted. This product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

8. Instructions for Use

When run in combination with assays with other fluorophores (channels), a Color Compensation file must be applied. To generate a Color Compensation file see instructions in the ***Roche 06296971001 Universal Color Compensation Hexaplex*** Instructions For Use.

8.1. Programming LightCycler® 480 Instruments

See the Instrument operator's manual for details. Start programming before preparing the solutions. The protocol consists of three program steps:

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification
- 3: Cooling: cooling the instrument

Detection Format 500 Channel — **Set Quant Factor 10, Max Integration Time 1 sec**

LightCycler® 480 Instrument: 450-500
LightCycler® 480 II Instrument: 440-488
cobas z 480 Analyzer (open channel): No filter combination for Cyan500 (opt. use FAM channel)

Program Step:	*RT Step**	*Denaturation*	*Cycling*			*Cooling*
Parameter						
Analysis Mode	*None*	None	Quantification mode			None
Cycles	*1*	1	45			1
Target [°C]	55	95	95	60	72	40
Hold [hh:mm:ss]	00:05:00	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	**Single**	None	None

* *optional use if combining with 1-Step RT-PCR*

Table 1

8.2. Experimental Protocol

- **Sample material:** Use aqueous nucleic acid preparations (e.g. 'High Pure PCR Template Preparation Kit').
- **Negative control:** Always run at least one no-template control (NTC) - replace the template DNA with water.
- **Positive control:** Run a positive control - replace the template DNA with the provided control DNA.

For an increased sensitivity use 10 µl nucleic acid per 20 µl reaction, for sample types were inhibition may occur e.g. Fecal sample extracts, use 5 µl. For 10 µl reactions in 384 well plates use 5 µl /2.5 µl.

8.2.1. Preparation of Parameter-Specific Reagents (PSR, 96 reactions):

The reagent vial with a **orange** cap contains all primers and probe to run 96+ LightCycler® reactions.

Check for the colored pellet, then **add 50 µl** PCR-grade water, mix (vortex) and spin down.
For robotic pipetting the volume can be extended to 55 µl (signals will decrease by 10-20%).

►**Use 0.5 µl** reagent for a 20 µl PCR reaction.

8.2.2. Preparation of the Positive Control DNA

Add 160 µl RNase/DNase-free 10 mM Tris buffer pH 8 - 8.5 to the vial with the **black** cap, if using 10 µl sample volume add **320 µl**. Mix by pipetting up and down 10 times. If vortexing spin down to collect the solution. Store dissolved controls frozen. Use of Tris increases the stability in solution.

Notes: Opening this vial may cause contamination of the workspace. Pulse spin vial prior to opening.

►**Use 5 µl** positive control (≈ Cp 30) for a 20 µl PCR reaction (10 µl if using 10 µl sample volume).

8.2.3. Preparation of Standard Row

The target DNA is provided in 6 different quantities to yield from 10^1 to10^6 target molecules in 5 µl once resolved. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. **Add 40 µl** PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution ten times up and down.

►**Use 5 µl** standard for a 20 µl PCR reaction.

8.2.4. Preparation of the Reaction Mix

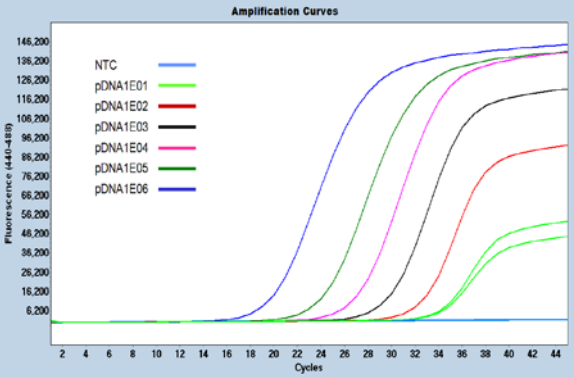
For use with the Roche LightCycler® Multiplex DNA Master		
One reaction	**Component**	**100 reactions**
5.5 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	550 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	50 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	400 µl
10.0 µl	**Volume of Reaction Mix**	**1,000 µl**

Table 2

Mix gently, spin down and **transfer 10 µl** per well.

Add 10 µl of sample or control DNA to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.
Start run

9. Typical Results (Data from LightCycler® 480 II system)

Channel 500 : CMV – single PCR	Positive Control
	

Dilution row 10E6 to 10 copies / reaction

Figure 1

10. Reading the Results

Perform data analysis as described in the operator's manual. For multiplex assays select the color compensation. We recommend using the Second Derivative Maximum method (Automated (F" max). View results in the 500 channel. The negative control (NTC) must show no signal.

Channel 500 (sample)	Channel 660 Control Reaction	Channel 500 NTC Control	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 40 +	**Not relevant**	**Negative**	**CMV Positive**
No amplification	**Not detectable**	**Not relevant**	**PCR failure Repeat**
Amplification signal	**Not relevant**	**Positive**	**Contamination Repeat**

+ Recommendation: Define the cut-off 2-4 cycles higher than observed Cp value for 10 copies.

11. References

12. Multiplex PCR Compatibility

This kit can be combined with other assays up to 6plex reactions including an internal control (IC) or a spiked extraction control (for example PhHV) as depicted below:

Note: For use with the z 480 Analyzer the CMV (500) results must be read in the FAM (530) channel. This requires to run a color compensation with the 500 reagent used for the FAM channel.

Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
CMV						X	X	X		
CMV	HSV-1	VZV	EBV	HSV-2	MSTN or	X				
CMV	HSV	VZV	EBV	HHV6	PhHV	X				
CMV	HSV	VZV	B19	Toxoplasma		X				

Table 3

13. Version History

V170830	Release Version	2017-08-30
V190123	Editorial changes 8.2.2 Use Tris buffer	2019-01-23

Certificate of Analysis (CoA)

Lot n°
Expiry :

Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range				29-32			
Measured							
Signal level							
Measured							
Negatives	10/10						✓

Note: Fluorescence (FL) levels depend on instrument settings and may vary. The crossing point (Cp) values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (ΔCp).

QC Acceptance Date:

We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.

Name(s) :

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