altona DIAGNOSTICS

RealStar®
CMV PCR Kit 1.0

06/2017

always a drop ahead.

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RealStar®

CMV PCR Kit 1.0

For research use only!

(RUO)

INS-021000-EN-02

REF Product No.: 021003

96 rxns

Store at -25°C ... -15°C

June 2017

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Content

The RealStar® CMV PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the detection and quantification of cytomegalovirus (CMV) specific DNA.

For research use only (RUO)! Not for use in diagnostic procedures.

1. Kit Components

Lid Color	Blue	Purple	Green	Red	White
Component	Master A	Master B	Internal Control	Quantification Standard*	PCR grade Water
Number of Vials	8	8	1	4	1
Volume [µl/Vial]	60	180	1000	250	500

*The RealStar® CMV PCR Kit 1.0 contains four different Quantification Standards (QS1- QS4)

2. Storage

- The RealStar® CMV PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage at +4°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

RealStar® CMV PCR Kit 1.0

3. Product Description

The RealStar® CMV PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the detection and quantification of CMV specific DNA. The reagent system includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

The test is based on real-time PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for CMV DNA are labelled with the fluorophore FAM. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE. Using probes linked to distinguishable dyes enables the parallel detection of CMV specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

Quantification of CMV specific DNA can be archieved by generation of a standard curve using external quantification standards (QS1, QS2, QS3, QS4).

The RealStar® CMV PCR Kit 1.0 can be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q 5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

NOTE

> ⚠ ***Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.***

4. Sample Preparation

Extracted DNA is the starting material for the RealStar® CMV PCR Kit 1.0. The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction kit is recommended for use with the RealStar® CMV PCR Kit 1.0:

- QIAamp® MinElute® Virus Spin Kit (QIAGEN)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid is highly recommended.

NOTE

> ⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***
>
> ⚠ ***Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

5. Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® CMV PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

- If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

- If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added depends always and only on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the sample/lysis buffer mixture.

NOTE

⚠ ***Never add the Internal Control directly to the sample!***

- If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

6. Reaction Setup

- Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Quantification Standard, Positive or Negative Control).
- Make sure that at least one Positive and one Negative Control are used per run.
- For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate lids.
- Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

7. Programming the Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® CMV PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support.

7.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX

7.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

Detection	Detector Name	Reporter	Quencher
CMV specific DNA	CMV	FAM	(None)
Internal Control	IC	JOE	(None)

7.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Denaturation	Hold	1	-	95 °C	10:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	58 °C	1:00 min

8. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument.

For detailed instructions regarding data analysis of the RealStar® CMV PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support.

9. Interpretation of Results

9.1 Qualitative Analysis

Sample ID	FAM Detection Channel	JOE Detection Channel	Result Interpretation
A	POSITIVE	POSITIVE*	CMV specific DNA detected.
B	NEGATIVE	POSITIVE	CMV specific DNA not detected. Sample does not contain detectable amounts of CMV specific DNA.
C	NEGATIVE	NEGATIVE	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE detection channel is not required for positive results in the FAM detection channel. High CMV load in the sample can lead to reduced or absent Internal Control signals.

9.2. Quantitative Analysis

The RealStar® CMV PCR Kit 1.0 provides four Quantification Standards (QS). In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with the appropriate concentrations (chapter 3. Product Description). Using **standards** of known concentrations a standard curve for quantitative analysis can be generated.

$$C_t = m \cdot \log (N_0) + b$$

C_t = Threshold Cycle
m = Slope
N_o = Initial Concentration
b = Intercept

Derived from the standard curve positive samples of unknown concentrations can be quantified.

$$N_0 = 10^{(Ct-b)/m}$$

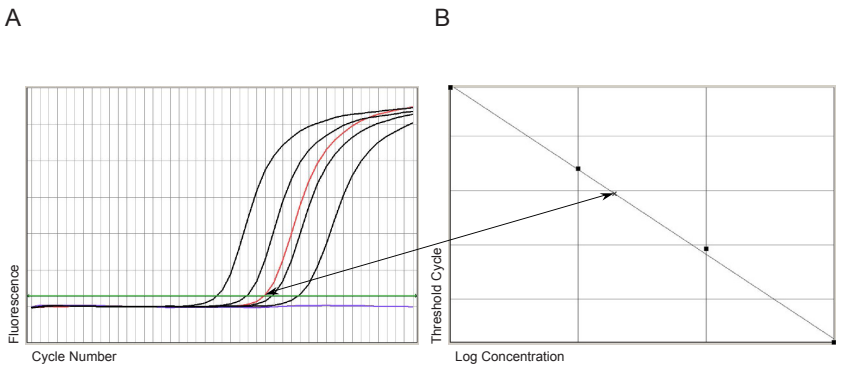

Figure 1: Quantification Standards (black), a positive (red) and a negative (blue) sample displayed in the Amplification Plot (**A**) and Standard Curve analysis (**B**).

NOTE

> ⚠ ***The concentration of your "Sample" is displayed in IU/µl and refers to the concentration in the eluate.***

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load } \textbf{(Sample)} \text{ [IU/ml]} = \frac{\text{Volume (}\textbf{Eluate}\text{) [µl] x Viral load (}\textbf{Eluate}\text{) [IU/µl]}}{\text{Sample Input [ml]}}$$

10. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

11. Trademarks and Disclaimers

RealStar® (altona Diagnostics GmbH); Mx 3005P™ (Stratagene); ABI Prism® (Applied Biosystems); LightCycler® (Roche); Rotor-Gene®; QIAamp®, MiniElute® (QIAGEN); VERSANT® (Siemens); CFX96™ (Bio-Rad).

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For research use only! Not for use in diagnostic procedures.

Not available in all countries.

12. Explanation of Symbols

Product number

Batch code


Contains sufficient for “n” tests/reactions (rxns)


Temperature limitation


Version


Use until


Caution


Consult instructions for use


Manufacturer

RealStar® CMV PCR Kit 1.0