



ANCHOR  
CMV PCR KIT

Instructions for Use  
ANCHOR CMV PCR Kit




Quantitative or Qualitative  
real-time PCR Kit  
for *in vitro* diagnostic use

**IVD** For *in vitro* diagnostic use


**REF** A1200

 100

 A1210-EN, 2025-04-29

**QG** A1211-EN, 2025-04-29

 -30°C to -15°C

 **ANCHOR** Diagnostics GmbH  
Grandweg 64  
D-22529 Hamburg

compatible with

QuantStudio 5 (Applied Biosystems)

LightCycler 480 II (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

Mic qPCR (Bio Molecular Systems)

1	▶ Contents	
2	Intended Use.....	6
3	Product Description.....	6
4	Kit Components.....	7
5	Storage and Stability.....	8
6	Material Required but Not Provided.....	9
7	Limitations.....	9
8	Warnings and Precautions .....	10
9	Workflow .....	11
9.1	Sample Preparation.....	11
9.1.1	Sample Matrix.....	11
9.1.2	Sample Preparation.....	12
9.1.3	Internal Control.....	13
9.2	PCR Preparation .....	14
9.2.1	Master Mix Set-Up.....	14
9.2.2	PCR Reaction Set-Up .....	16
9.3	PCR Cycler Configuration .....	17
9.3.1	Temperature Profile .....	17
9.3.2	Specific PCR Cycler Settings .....	18
9.4	Data Analysis .....	20
9.4.1	Qualitative Analysis.....	22
9.4.2	Quantitative Analysis.....	23
10	Performance Data.....	24
10.1	Analytical Performance.....	24
10.1.1	Sensitivity.....	24
10.1.2	Linearity.....	25
10.1.3	Specificity.....	26
10.1.4	Precision .....	28
10.2	Clinical Performance.....	30
11	Quality Control.....	30
12	Technical Assistance & Contact Information .....	31
13	Literature.....	31
14	Symbols .....	32

## 2 ▶ Intended Use

The ANCHOR CMV PCR Kit is an *in vitro* nucleic acid amplification test, based on real-time PCR technology, for the quantitative or qualitative detection of CMV (Human betaherpesvirus 5) DNA, isolated from human EDTA plasma in combination with the Rotor-Gene Q, LightCycler 480 II, CFX96, Mic qPCR and QuantStudio 5 real-time PCR instruments.

The ANCHOR CMV PCR Kit is not intended for use as a screening test for blood and blood products or for screening purposes in prematernity and organ transplant settings.

The product is intended to be used by professional users, such as laboratory technicians and physicians who are trained in molecular biological techniques.

## 3 ▶ Product Description

The Kit constitutes a ready-to-use system for the amplification, detection and quantitation of CMV-specific nucleic acids.

In addition, a heterologous amplification system (Internal Control) is included to supervise the success of the sample extraction procedure and to identify possible inhibition of the amplification reaction.

Probes linked to distinguishable fluorescent dyes enable the parallel detection of CMV specific nucleic acids and the Internal Control in two corresponding detector channels of the real-time PCR instrument.

The Quantitation Standards QS1-4 CMV contain defined concentrations of artificial DNA bearing the CMV target sequence. They are calibrated against the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code 09/162) and can be used individually or as a whole set together with the Negative Control DNA to monitor the integrity of the analyte-specific reagents of the kit and the proper performance of the reaction. When the Quantitation Standards QS1-4 CMV are used as a whole set, they allow to quantitate the CMV DNA present in a test sample.

## 4 ▶ Kit Components

Master A and Master B reagents contain all necessary components to allow PCR-mediated amplification and target detection of CMV specific DNA and Internal Control in one reaction setup.

The Quantitation Standards QS1-4 CMV and NC (Negative Control) DNA are supplied with the IC (Internal Control) DNA already incorporated (see also section 9.2.1 Master Mix Set-Up).

The reagents provided with the kit allow the preparation of 100 reactions.

Kit component	Mat. no.	No. of vials	Quantity	Ingredients
<b>Master A CMV</b>	A1201	4 vials	4x 125 µL	Buffer, bovine serum albumin, polymerase
<b>Master B CMV</b>	A1202	4 vials	4x 125 µL	Buffer, salt, nucleotides, target- and IC-specific oligonucleotides
<b>IC DNA</b>	AD00020	1 vial	1000 µL	Buffer, IC-specific synthetic polynucleotide
<b>QS1-4 CMV*</b>	A1203- 1/2/3/4	1 vial each	4x 200 µL	Buffer, target- and IC-specific synthetic polynucleotide
<b>NC DNA*</b>	AD00021	1 vial	200 µL	Buffer, IC-specific synthetic polynucleotide

**\* INTERNAL CONTROL INSIDE !**

## 5 ▶ Storage and Stability

- The ANCHOR CMV PCR Kit is shipped on dry ice and should be stored at -30 to -15°C upon receipt.
- The components are stable until the expiration date stated on the label.
- Do not use components of the kit that have passed their expiration date.
- Store CMV DNA-positive and/or potentially positive material separated from the kit.
- Repeated thawing and freezing of the Master reagents of > 3x should be avoided, as this may reduce the assay performance. For the Quantitation Standards QS1-4 CMV, the NC DNA and the IC DNA, thawing and freezing cycles up to 4x are allowed. Alternatively, storage between +2 to +8°C for up to 14 days is possible.
- Due to the components used it might be possible that Master vials do not always freeze completely after initial thawing. This is not a matter of concern and does not influence the stability or performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Label aliquots clear and unambiguously to avoid a mix-up of reagents.
- During PCR set up the reagents should be kept cooled at +2 to +8°C – use cooling block.
- Do not store Master A and Master B CMV more than 3 h at +2 to +8°C.
- Protect all reagents from extensive light exposure.

## 6 ▶ Material Required but Not Provided

- Nucleic acid purification system
- Real-time PCR instrument
- Appropriate PCR reaction vessels and related accessories
- Cooling block (for reaction setup)
- Benchtop centrifuge (rotor holding 2 mL reaction tubes)
- Vortex mixer
- Pipettes (variable volume)
- Single-use pipette filter tips
- 1.5 mL or 2 mL reaction tubes (for Master mix set-up)
- Single-use gloves (powder-free)

Use all materials and equipment according to the manufacturer's instructions. Maintain and calibrate the equipment as recommended.

## 7 ▶ Limitations

- Strict compliance with the user manual is required for optimal PCR results.
- Any diagnostic result generated must be interpreted in conjunction with other clinical and/or laboratory findings.
- The presence of PCR inhibitors may cause invalid results.
- Occurrence of mutations within the target region might result into a reduced sensitivity, false quantitation or a complete detection failure.
- Following good laboratory practices is crucial for the successful usage of the product.
- Appropriate handling of the reagents is essential to avoid contaminations or impurities.

## 8 ▶ Warnings and Precautions

- For *in vitro* diagnostic use.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as potentially infectious and/or biohazardous material in accordance with safe laboratory procedures.
- The ANCHOR Master A CMV contains a bovine-sourced potentially infectious component (albumin). The bovine plasma is sourced from New Zealand or USA, which are recognized by the world organization for animal health *Office International des Epizooties* (OIE, Paris) as having a negligible BSE risk.
- Wear protective single-use gloves, a laboratory coat and eye protection when handling specimens or kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free single-use pipette tips with aerosol barriers.
- Use separated working areas for (1) specimen preparation, (2) PCR reaction set-up and (3) amplification/detection activities.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Discard sample and assay waste according to your local safety regulations.

## 9 ▶ Workflow

### 9.1 ▶ Sample Preparation

#### 9.1.1 ▶ Sample Matrix

The recommended patient sample matrix for sample preparation input is:

- Human EDTA plasma

The blood draw should be done using commercially available standard blood collection systems for EDTA plasma (e.g. Sarstedt, Becton Dickinson, Greiner or equivalent). Tube contents should be mixed directly after sample collection according to manufacturer's instructions. For separation of EDTA plasma, whole blood should be centrifuged according to the instructions provided by the manufacturer of the collection system within 24 hours after collection.

If a sample is not directly processed, store samples according to manufacturer's instructions (Collection Tube, Transport Tube, Sample Preparation Kit) before use. For shipment, follow the local and national regulations for the transport of biological material.

Storage recommendations:

If not specified by related manufacturer's instructions - storage of clinical samples under refrigerated conditions (+2 °C to +8 °C) should not exceed 14 days (Abdul-Ali et al., JCV, 2011).

### 9.1.2 ▶ Sample Preparation



The diagnostic applicability of the ANCHOR CMV PCR Kit was evaluated and confirmed using the following sample preparation platform:

#### Sample Preparation Platform

GenoXtract 96 System (Hain Lifescience GmbH)

Purified DNA is the sample input material for the ANCHOR CMV PCR Kit. It has to be ensured by the user that the chosen nucleic acid purification system is compatible with real-time PCR technology. Extract the nucleic acids according to the manufacturer's instructions.


#### PLEASE NOTE

-  If sample eluates are not directly used for PCR analysis, store eluates at -30 to -15 °C. In case of using eluates repeatedly, avoid frequent thaw/freeze cycles (no more than 3 cycles).
-  Eluates should be labeled clearly and unambiguously to avoid a mix-up of samples.

### 9.1.3 ▶ Internal Control

The Internal Control DNA provided with the ANCHOR CMV PCR Kit should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality.

#### PLEASE NOTE

-  The Internal Control DNA **MUST NOT** be added directly to the clinical sample.

Always add the Internal Control DNA after lysis buffer has been added to the sample.


The required volume of Internal Control DNA per sample purification is defined by the chosen elution buffer volume.

Ten percent of the elution buffer volume used should be added to the sample/lysis mixture.

Examples:

- Elution buffer per sample: 200 µL → IC DNA volume: 20 µL
- Elution buffer per sample: 60 µL → IC DNA volume: 6 µL

#### PLEASE NOTE

-  Secure the elimination of residual ethanol before elution of nucleic acids. Ethanol may inhibit the amplification process.

If no co-purification of the Internal Control is planned and the IC DNA should be used only as an inhibition control of the reaction, either the amount of IC related to the used elution volume could be added to each eluate or 1.5 µL of the IC DNA per reaction should be added to the master mix (see section 9.2.1 Master Mix Set-Up).

9.2 ▶ PCR Preparation  
 9.2.1 ▶ Master Mix Set-Up

**PLEASE NOTE**



Consider configuring the run settings of the PCR cycler software to have the instrument ready before starting the PCR reaction preparation (Refer to section 9.3 PCR Cycler Configuration).

Prepare the Master Mix step by step:

- Thoroughly thaw Master components A and B.
- Mix Master A and B by gentle pipetting or short pulse-vortexing.
- Spin Master A and B shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- According to your preferred workflow follow one of the pipette schemes below to mix Master A and B using a 1.5 mL or 2 mL reaction tube:

**IC DNA present in sample eluates** – NO IC DNA added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A CMV	5 µL (X)	55 µL	Y µL
Master B CMV	5 µL (X)	55 µL	Y µL
Volume Master Mix	10 µL	110 µL	Z µL

\*10 reactions + 10%

\*\* See formula on next page

**IC DNA to be used as inhibition control only** – IC DNA added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A CMV	5 µL (X)	55 µL	Y µL
Master B CMV	5 µL (X)	55 µL	Y µL
IC DNA	1.5 µL (X)	16.5 µL	Y µL
Volume Master Mix	11.5 µL	126.5 µL	Z µL

\*10 reactions + 10%

\*\* See formula on next page

**PLEASE NOTE**



We recommend calculating for an additional volume of at least 10% to compensate potential loss during pipetting. The needed volume will be calculated by using the following formula:

$$N \times X \mu L \times 1,1 = Y$$

N = Number of reactions

X = Volume of component per reaction

Y = Total volume of component

Z = Total volume of Master Mix

- Mix prepared Master Mix by gentle and short pulse-vortexing.
- Spin Master Mix shortly with a benchtop centrifuge to remove residual droplets from tube lids.



It is recommended to test all 4 Quantitation Standards and the Negative Control at least once in each PCR run for quantitative purposes. For qualitative analyses, the use of QS3 CMV as Positive Control is recommended. For further information, see also chapters 9.4.1 and 9.4.2, respectively.



Quantitation Standards QS1-4 CMV and the Negative Control DNA already contain the IC DNA in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC DNA (as inhibition control) in combination with the QS1-4 CMV and NC DNA, be aware that the IC signal of the controls will slightly shift towards a lower CT value in comparison to the IC signal of the controls using a Master mix without additional IC.

9.2.2 ▶ PCR Reaction Set-Up

**PLEASE NOTE**

**i** Always use a cooling block for the preparation of the PCR reaction mix.

Prepare the Reaction Mix step by step:

- If previously stored frozen, thaw eluates containing nucleic acid (and IC DNA) thoroughly.
- Mix eluates by gentle pipetting or brief pulse-vortexing.
- Spin eluates shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- Pipette **10 µL of Master Mix** (see section 9.2.1 Master Mix Set-Up) into suitable reaction vessels for PCR analysis. This is also valid for Master Mix spiked with IC DNA .
- Add **15 µL of eluate** or control (Quantitation Standards QS1-4 CMV or Negative Control DNA).  
**Mix well by repeated up and down pipetting.**
- Close reaction vessels securely with the appropriate sealing system.
- Immediately transfer closed and ready-to-use reaction vessels to the real-time PCR instrument. Avoid any delays!

**PLEASE NOTE**

**i** Carefully handle reaction vessels during transfer to avoid mixing up samples.

**i** Complete mixing of Master Mix reagents with a sample or control during reaction set up should be unconditionally secured by repeated up and down pipetting!

**This is essential for achieving optimal amplification curve performance.**

Master Mix	Eluate / Control	Reaction Mix
10 µL	15 µL	25 µL

9.3 ▶ PCR Cycler Configuration

The ANCHOR CMV PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 28 min.
LightCycler 480 II (Roche)	≈ 30 min.
CFX96 (Bio-Rad)	≈ 33 min.
Rotor-Gene Q (QIAGEN)	≈ 39 min.
Mic qPCR (Bio Molecular Systems)	≈ 33 min.

The listed run times for the different instruments are effectively measured durations and can differ from what is displayed on the graphical user interface of the individual instrument software. For basic information concerning set-up and programming of the respective real-time PCR instrument, refer to the instrument-specific manual.

9.3.1 ▶ Temperature Profile

Temperature cycling profile for **QuantStudio 5, LightCycler 480 II, CFX96** and **Rotor-Gene Q**:

Cycling	95°C	1 sec	x 40
	65°C *	2 sec	
	72°C	1 sec	

\* Fluorescence acquisition for CMV and IC

Temperature cycling profile for **Mic qPCR**:

Cycling	95°C	1 sec	x 40
	63°C *	2 sec	
	72°C	1 sec	

\* Fluorescence acquisition for CMV and IC

Reaction Volume: 25 µL

9.3.2 ▶ Specific PCR Cycler Settings

The following tables contain PCR cycler-specific recommendations for the basic configuration of the run settings.

For additional information regarding the cycler settings recommended plastics, color compensation, gain optimization settings, etc. do not hesitate to contact us directly (see section 12 Technical Assistance & Contact Information).

QuantStudio™ 5		
Target	CMV	IC
Detection	FAM	HEX
Run Settings <ul style="list-style-type: none"> <li>Block Type: 96-Well 0.1-mL Block</li> <li>Experiment Type: Standard Curve Chemistry: TaqMan® Reagents</li> <li>Run Mode: Fast</li> <li>Plate attributes: Passive Reference - None</li> </ul>		
Consumables: <ul style="list-style-type: none"> <li>96-Well Fast Thermal Cycling Plates (Life Technologies Mat.No. 4346907)</li> <li>MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971)</li> </ul>		

LightCycler® 480 II		
Target	CMV	IC
Detection	465/510	533/580
Run Settings: <ul style="list-style-type: none"> <li>Block size: 96</li> <li>If clear plates are used, the sensor of the LightCycler® has to be disabled by selecting the Clear Plates option in the software before the run is started.</li> </ul>		
Consumables: <ul style="list-style-type: none"> <li>LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001)</li> <li>LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001)</li> <li>LC480 Sealing Foil (Roche Mat. No. 04729757001)</li> </ul>		

Bio-Rad CFX96		
Target	CMV	IC
Detection	FAM	HEX
Consumables: <ul style="list-style-type: none"> <li>Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655)</li> <li>Optical flat 8 Cap Strip for 0.2mL (Mat. No. TCS0803)</li> <li>0.2 mL 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad Mat. No. TLS 0851)</li> <li>8-strip optical clear flat caps (Sarstedt Mat. No.65.1998.400)</li> </ul>		

Rotor-Gene Q		
Target	CMV	IC
Detection	Green	Yellow
Run Settings <ul style="list-style-type: none"> <li>Use 72-Well Rotor</li> <li>Perform Auto-Gain optimization before 1st acquisition.</li> </ul>		
Consumables: <ul style="list-style-type: none"> <li>Strip Tubes and Caps, 0.1 mL (QIAGEN Mat. No 981103)</li> </ul>		

Mic qPCR		
Target	CMV	IC
Detection	Green	Yellow
Run Settings <ul style="list-style-type: none"> <li>Temperature Control: Standard TAQ</li> </ul>		
Consumables: <ul style="list-style-type: none"> <li>Mic Tubes and Caps (Mat. No.68MIC-60653)</li> </ul>		

When preferring a quantitative analysis of the clinical samples, the Quantitation Standards QS1-4 CMV must be labeled as standards within the instrument software and assigned with their appropriate concentrations.

Quantitation Standard	Concentration [IU/μL]
QS1 CMV	50,000
QS2 CMV	5,000
QS3 CMV	500
QS4 CMV	50

#### 9.4 ▶ Data Analysis

The following tables contain cycler-specific references for the configuration of analysis settings. They could serve as an initial orientation. Depending on local cycler- and workflow-related differences adaptations might be necessary.

For additional information concerning data analysis, refer to the instrument-specific manual of the respective real-time PCR instrument or contact us (see section 12 Technical Assistance & Contact Information).

QuantStudio™ 5
Analysis Settings (all channels): <ul style="list-style-type: none"> <li>▪ Plot Type: ΔRn vs Cycle</li> <li>▪ Graph Type: Linear</li> <li>▪ Baseline Start/End: 3/15</li> <li>▪ Threshold:                             <ul style="list-style-type: none"> <li>- FAM 300,000</li> <li>- HEX 70,000</li> </ul> </li> </ul>

LightCycler® 480 II
Analysis Settings: <ul style="list-style-type: none"> <li>▪ Abs Quant/Fit Points</li> <li>▪ Color Comp (off)</li> <li>▪ Mean</li> <li>▪ High Confidence</li> <li>▪ Threshold:                             <ul style="list-style-type: none"> <li>- 465/510: 2.0</li> <li>- 533/580: 2.5</li> </ul> </li> </ul>

Bio-Rad CFX96
Analysis Settings (all channels): <ul style="list-style-type: none"> <li>▪ Baseline Subtracted Curve Fit</li> <li>▪ C(t) Determination Mode: Single Threshold</li> <li>▪ Baseline Threshold:                             <ul style="list-style-type: none"> <li>- Baseline Cycles: Auto Calculated</li> <li>- Single Threshold                                     <ul style="list-style-type: none"> <li>- FAM: 1,000</li> <li>- HEX: 350</li> </ul> </li> </ul> </li> </ul>

Rotor-Gene Q
Analysis Settings (all channels): <ul style="list-style-type: none"> <li>▪ Quantitation</li> <li>▪ Linear Scale</li> <li>▪ Dynamic Tube ON</li> <li>▪ Threshold:                             <ul style="list-style-type: none"> <li>- Green: 0.5</li> <li>- Yellow: 0.8</li> </ul> </li> </ul>

Mic qPCR
Analysis Settings (all channels): <ul style="list-style-type: none"> <li>▪ Graph Type: Linear</li> <li>▪ Method: Dynamic</li> <li>▪ Ignore Cycles Before: 3</li> <li>▪ Threshold Start: 1</li> <li>▪ Exclusion: None</li> <li>▪ Threshold Level:                             <ul style="list-style-type: none"> <li>- Green: 1.0</li> <li>- Yellow: 1.4</li> </ul> </li> </ul>

9.4.1 ▶ Qualitative Analysis

For a valid run and as a prerequisite for the interpretation of the individual clinical sample results, the following requirements have to be met by the included kit controls:

Channel/Target	CMV	IC
QS3 CMV <sup>1</sup>	+	+
NC DNA	-	+

<sup>1</sup> It is recommended to use QS3 CMV as Positive Control

If one of the conditions has failed, result interpretation of clinical sample results might be flawed. In case of kit control failure, it is recommended to repeat the PCR run.

In case of a valid run, the following result interpretation can be made:

Qual. result	CMV	IC
CMV DNA Positive	+	+/-
CMV DNA Negative	-	+
Invalid	-	-

A positive result for CMV DNA does not necessarily require a positive signal for the IC since high concentrations of the respective target nucleic acid can result in a competitive inhibition of the IC amplification.

An invalid result for a clinical sample can be due to PCR inhibition or a failure during the nucleic acid isolation procedure. In such cases, it is recommended to dilute the nucleic acid extract 1:10 (recommended to be done in elution buffer, if possible) for a PCR retest or to repeat the nucleic acid isolation procedure. Note that the dilution of the nucleic acid extract might also lead to a reduction of the target nucleic acid concentration below the limit of detection of the ANCHOR CMV PCR Kit.

9.4.2 ▶ Quantitative Analysis

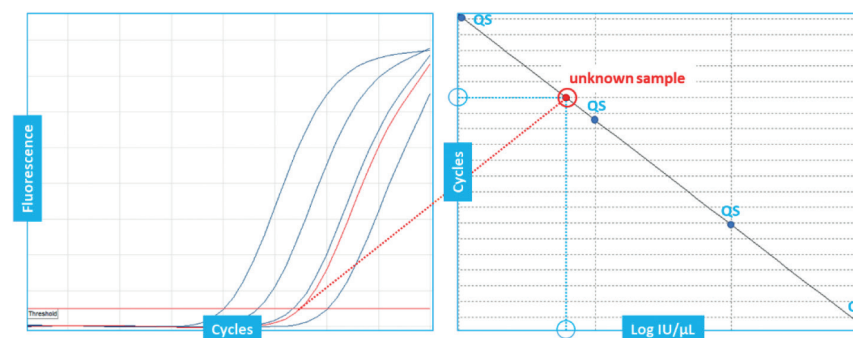
For a valid run and as a prerequisite for the interpretation of the individual clinical sample results, the following requirements have to be met by the included kit controls:

Channel/Target	CMV	IC	Correlation Coefficient r <sup>2</sup>	Slope m
QS1-4 CMV	+	(+) <sup>2</sup>	≥0.99	-3.0 to -3.6
NC DNA	-	+	-	-

<sup>2</sup> QS1 CMV is excluded from this rule. The presence of high concentrated artificial nucleic acids in this Standard can result in a competitive inhibition of the IC amplification.

If one of the conditions has failed, the quantitative interpretation of the clinical sample results might be flawed. In such cases, it is recommended to repeat the PCR run.

If all criteria are met, the standard curve generated with QS1-4 CMV of known concentrations can be used to determine the CMV DNA load present in a clinical sample.



The concentration of any target DNA within a sample eluate will be quantified according to the formula

$$Conc. = 10^{\frac{C_t - b}{m}}$$

where *m* is the slope of the standard curve and *b* the y-intercept.

The results are displayed in IU/μL. To calculate the concentration of CMV DNA in the original clinical sample in IU/mL, the concentration factor of the applied sample preparation system must be considered:

$$\text{Sample} \frac{\text{IU}}{\text{mL}} = \text{Eluate} \frac{\text{IU}}{\mu\text{L}} \times \frac{\text{Volume Eluate} [\mu\text{L}]}{\text{Volume Sample input [mL]}}$$

For inter-assay result comparison, a conversion factor needs to be established to adjust quantitative results considering workflow-specific performance characteristics. If you plan to establish such a conversion factor please contact us for technical assistance, if required (see section 12 Technical Assistance & Contact Information).

10 ▶ Performance Data

10.1 ▶ Analytical Performance

10.1.1 ▶ Sensitivity

The LOD for the ANCHOR CMV PCR Kit was determined by undertaking a probit analysis on the Rotor-Gene Q platform. A dilution series of different concentration levels for CMV strain Merlin DNA (NIBSC 09/162) was used. Each dilution level was tested with overall 24 replicates using 3 different PCR reagent lots across 3 different days, executed by 2 different persons on 2 different instruments.

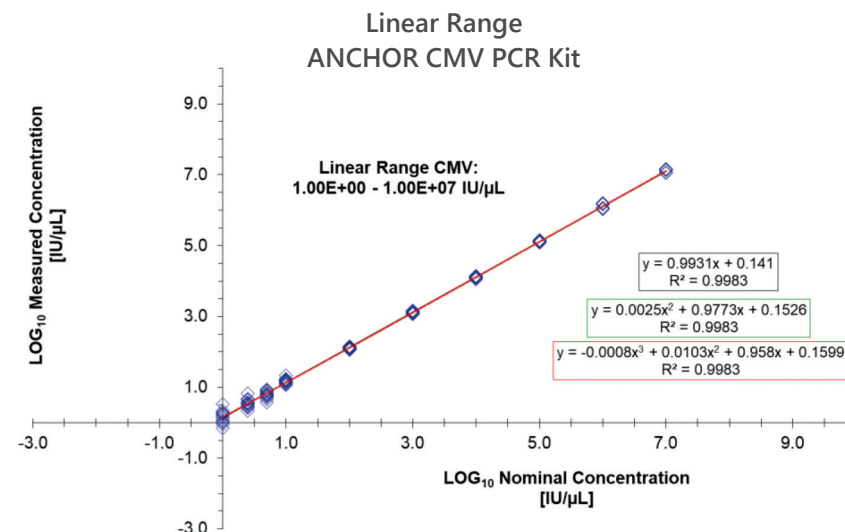
The LOD value determined on the Rotor-Gene Q was then confirmed or re-evaluated on the other 4 instruments.

Instrument	LOD	Unit
Rotor-Gene Q	0.66	IU/μL
QuantStudio 5	0.66	IU/μL
LightCycler 480 II	0.66	IU/μL
CFX96	0.66	IU/μL
Mic qPCR	0.66	IU/μL

10.1.2 ▶ Linearity

The linear range of the ANCHOR CMV PCR Kit was determined by testing a dilution series of artificial DNA comprising the CMV PCR target, ranging from 1.00E+07 IU/μL to 1.00E+00 IU/μL. The dilutions were analysed concentration-dependent with 4-8 replicates. All replicates of one dilution were tested in one PCR run. The linear range was determined with two ANCHOR CMV PCR Kit lots.

The picture below shows a scatter chart of measured concentrations plotted against their nominal concentrations. The correlation of data is described by linear, 2nd and 3rd order polynomial curve fitting.



The linear quantification range for the ANCHOR CMV PCR Kit was tested and confirmed to be from at least 1.00E+00 up to 1.00E+07 IU/μL. The Limit of Quantitation (LOQ) is therefore determined to be 1.00E+00 IU/μL.

10.1.3 ► Specificity

Triplicates of four different CMV culture strains were tested at a concentration near the 3x LOD of the ANCHOR CMV PCR Kit.

Strain	CMV
CMV strain Merlin	+
CMV strain AD-169	+
CMV strain Towne	+
CMV strain Davis	+

Nucleic acid of selected pathogens with a concentration of  $\approx 5.00E+03$  copies/ $\mu\text{L}$  (alternative units CFU/ $\mu\text{L}$  or TCID<sub>50</sub>/ $\mu\text{L}$ ) was added to the PCR reaction and tested in triplicates in the absence or presence of CMV DNA at its 3x LOD and 3x LOQ concentration on the Rotor-Gene Q.

Pathogen	- CMV	3x LOD CMV	3x LOQ CMV $\Delta \text{Log}_{10} \text{IU}/\mu\text{L}^3$
Adenovirus 2	-	+	0.05
Adenovirus 4	-	+	-0.06
Adenovirus 5	-	+	-0.16
HSV-1	-	+	-0.10
HSV-2	-	+	0.00
BK Virus	-	+	-0.14
JC Virus	-	+	-0.03
Epstein-Barr Virus	-	+	-0.11
Hepatitis A Virus	-	+	-0.09
Hepatitis B Virus	-	+	-0.13
Human Herpesvirus 6A	-	+	-0.20
Human Herpesvirus 6B	-	+	-0.10
Human Herpesvirus 7	-	+	-0.08
Human Herpesvirus 8	-	+	0.13
Human Parvovirus B19	-	+	0.12
Enterovirus 71	-	+	0.08
West-Nile Virus	-	+	0.08
VZV	-	+	0.14
<i>Candida albicans</i>	-	+	0.00
<i>Aspergillus fumigatus</i>	-	+	0.01
<i>Aspergillus niger</i>	-	+	0.08
<i>Salmonella typhimurium</i>	-	+	0.06
<i>Streptococcus pneumoniae</i>	-	+	0.10
<i>Cutibacterium acnes</i>	-	+	0.07
<i>Staphylococcus aureus</i>	-	+	0.00

<sup>3</sup> In relation to CMV DNA-only control

10.1.4 ► Precision

Precision testing was initially performed on the Rotor-Gene Q instrument. For intra-run variability, 3-6 replicates of each sample dilution were tested within one run using one instrument and reagent lot by one operator. For inter-run variability, 3-6 replicates of each sample dilution were tested within overall four runs using two instruments and one reagent lot by two operators across days. For inter-batch variability, 3-5 replicates of each sample dilution were tested within one run using one instrument and three reagent lots by one operator.

QS1 CMV			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	20.18	0.05	0.23
Inter-Run	20.14	0.13	0.64
Inter-Batch	20.20	0.17	0.85
Total	20.16	0.16	0.79
QS4 CMV			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	30.26	0.07	0.23
Inter-Run	30.34	0.21	0.69
Inter-Batch	30.26	0.19	0.63
Total	30.31	0.22	0.71
QS CMV 3xLOQ (3 IU/μL)			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	34.39	0.41	1.20
Inter-Run	34.80	0.56	1.60
Inter-Batch	34.46	0.43	1.23
Total	34.71	0.54	1.55

WHO Int. Stand. CMV 3xLOQ (3 IU/μL)			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	33.81	0.23	0.69
Inter-Run	33.71	0.32	0.95
Inter-Batch	33.86	0.34	1.01
Total	33.76	0.35	1.03
QS CMV 3xLOQ (3 IU/μL)			
Variability	AVE (IU/μL)	SD (IU/μL)	CV (%)
Intra-Run	3.04	0.88	28.81
Inter-Run	2.67	1.05	39.45
Inter-Batch	2.67	0.80	30.02
Total	2.61	0.96	36.77
WHO Int. Stand. CMV 3xLOQ (3 IU/μL)			
Variability	AVE (IU/μL)	SD (IU/μL)	CV (%)
Intra-Run	4.42	0.73	16.52
Inter-Run	5.42	1.72	31.76
Inter-Batch	3.98	0.92	23.10
Total	4.92	1.70	34.57

Precision of the ANCHOR CMV PCR Kit in combination with the other instruments was evaluated for intra- and inter-run variability.

10.2. ▶ Clinical Performance

The clinical performance of the ANCHOR CMV PCR Kit for the qualitative and quantitative detection of CMV DNA in human EDTA plasma samples was evaluated comparatively at one study site against an established CMV routine diagnostic workflow using a CE-marked PCR-Assay as reference standard.

415 prospectively collected specimen were analysed with the ANCHOR CMV PCR Kit and with the comparator assays to determine their positive percent agreement (PPA) and negative percent agreement (NPA), respectively. Testing was done using the CFX96 PCR Cyclor.

ANCHOR CMV PCR Kit	Σ 415	Comparator	
		POS	NEG
POS	78	16	
NEG	16	305	

PPA: 83.0 %    NPA: 95.0 %

11 ▶ Quality Control

In accordance with the implemented ISO 13485-certified Quality Management System, each lot of the ANCHOR CMV PCR Kit is tested against predetermined specifications to ensure consistent product quality.

12 ▶ Technical Assistance & Contact Information















For any questions, a need for technical assistance or if you identify difficulties using our products do not hesitate to contact us:

phone: +49 40 52 471 62 0  
 email: [support@anchor-diagnostics.com](mailto:support@anchor-diagnostics.com)

13 ▶ Literature

- (1) Griffiths et al., The pathogenesis of human cytomegalovirus. *J Pathol* 2015; 235: 288-297.
- (2) Dioverti et al., Cytomegalovirus. *Microbiol Spectrum* 4(4):DMIH2-0022-2015.
- (3) Britt et Prichard, New therapies for human cytomegalovirus infections. *Antiviral Research* (2018).
- (4) Drozd et al., Cutaneous cytomegalovirus manifestations, diagnosis, and treatment: a review. *Dermatology Online Journal*, Vol 25, No 1, January 2019, 25 (1):2.
- (5) Haidar at Singh, Viral infections in solid organ transplant recipients: novel updates and a review of the classics. *Co-Infectious Diseases*, 2017.
- (6) Kotton et al., The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation. *Transplantation* 2018;102: 900–931.
- (7) Fryer et al., A collaborative study to establish the 1st WHO International Standard for human cytomegalovirus for nucleic acid amplification technology. *Biologicals*, Vol 44, Issue 4, July 2016, 242-251.
- (8) Walston et al., Clinical Guideline: Diagnosis and Management of Congenital Cytomegalovirus. *East of England CMV guideline* 2017.
- (9) Razonable et Humar, Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant*. 2019 Sep; 33(9).
- (10) Khalil et al., Congenital Cytomegalovirus Infection: Update on Treatment. *Royal College of Obstetricians and Gynecologists*. No 56 *BJOG* 2018, 125:e1-e11.
- (11) Razonable et Hayden, Clinical Utility of Viral Load in Management of Cytomegalovirus Infection after Solid Organ Transplantation. *Clin Microbiol Rev*. 2013 Oct; 26(4): 703–727.
- (12) Azedevo et al., Cytomegalovirus infection in transplant recipients. *Clinics (Sao Paulo)*. 2015 Jul; 70(7): 515–523.
- (13) Kraft et al., Interpreting Quantitative Cytomegalovirus DNA Testing: Understanding the Laboratory Perspective. *Clin Infect Dis*. 2012 Jun 15; 54(12): 1793–1797.
- (14) Wilkinson et al., Human cytomegalovirus: taking the strain. *Med Microbiol Immunol*. 2015; 204(3): 273–284.
- (15) Abdul-Ali et al., Cytomegalovirus DNA stability in EDTA anti-coagulated whole blood and plasma samples. *Journal of Clin. Virol*. 2011 Nov; 52(3): 222-224.

## 14 ▶ Symbols

-  Component in Kit
-  Volume per vial
-  For *in vitro* diagnostic use
-  Batch code
-  Number of vials
-  Quick Guide - Catalog number and version
-  Product - Catalog number
-  Unique Device Identifier
-  Catalog number and version  
Consult Instructions for Use
-  Important Note
-  Use by
-  Contains sufficient reagents for <N> tests
-  Temperature limits for storage
-  Manufacturer



Grandweg 64  
22529 Hamburg | Germany  
phone: +49 40 52 471 62 0  
[www.anchor-diagnostics.com](http://www.anchor-diagnostics.com)