ANCHOR
DIAGNOSTICS

ANCHOR
HHV-6/7/8 PCR Kit

Instructions for Use

ANCHOR HHV-6/7/8 PCR Kit

RUO

Quantitative or Qualitative

Real-Time PCR Kit

For Research Use Only

RUO	For Research Use Only
REF	AD02300
Σ	100
	AD02390-EN, 2025-11-19
QG	AD02391-EN, 2025-11-11
	-30°C to -15°C
	ANCHOR Diagnostics GmbH Grandweg 64 D-22529 Hamburg

Instructions for Use – ANCHOR HHV-6/7/8 PCR Kit

compatible with

QuantStudio 5 / QuantStudio 5 Dx (Applied Biosystems)

cobas z480 Analyzer (Roche)

LightCycler PRO (Roche)

CFX 96 Deep Well Dx / CFX Opus 96 Dx (Bio-Rad)

Rotor-Gene Q (QIAGEN)

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2 ▸ Product Description

The ANCHOR HHV-6/7/8 PCR Kit is a real-time PCR technology based test for the amplification, detection and differentiation of nucleic acids from Human Herpesvirus (HHV) 6 (DNA polymerase), HHV-7 (U56), and HHV-8 (ORF68).
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 and differentiation of Human Herpesvirus 6, 7 and 8 nucleic acids and the Internal Control (IC) in four corresponding detector channels of the real-time PCR instrument. The Quantification Standards (QS) contain artificial DNA bearing the HHV-6, -7 and -8 target sequences. They can be used together with the Negative Control DNA to monitor the integrity of the analyte-specific reagents of the kit and the proper performance of the amplification

3 ▸ Kit Components

Master A and Master B reagents contain all necessary components (PCR buffer, Polymerase, magnesium ions, dNTPs, primers and probes) to allow PCR mediated amplification and target detection of HHV-6, -7 and -8 specific DNA and Internal Control in one reaction setup.
The QS (Quantification Standards) and NC (Negative Control) DNA are supplied with the IC (Internal Control) DNA already incorporated (see also section 8.2.1 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
Master A HHV-6/7/8	AD02308	4 vials	4x 125 µL	Buffer, bovine serum albumin, polymerase
Master B HHV-6/7/8	AD02309	4 vials	4x 125 µL	Buffer, salt, nucleotides, target- and IC-specific oligonucleotides
IC DNA	AD00020R	1 vial	1000 µL	Buffer, IC-specific synthetic polynucleotide
QS1-4 HHV-6/7/8*	AD0230-1/2/3/4	1 vial each	200 µL	Buffer, target- and IC-specific synthetic polynucleotide
NC DNA*	AD00021R	1 vial	200 µL	Buffer, IC-specific synthetic polynucleotide

*INTERNAL CONTROL INSIDE!

4 ▸ Storage and Stability

- The ANCHOR HHV-6/7/8 PCR Kit is shipped on dry ice and should be stored at -30 to -15°C upon receipt.
- Store HHV-6, -7 and/or -8 DNA-positive and/or potentially positive materials 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 HHV-6/7/8, 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 HHV-6/7/8 more than 3 h at +2 to +8°C.
- Protect all reagents from extensive light exposure.

5 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 by the manufacturer.

6 Limitations

- Strict compliance with the Instructions for Use is required for optimal PCR results.
- The presence of PCR inhibitors may cause invalid results.
- Occurrence of mutations within the target region might result in a reduced sensitivity 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.

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7 Warnings and Precautions

- For research use only. Not for use in diagnostic procedures.
- Use of this product is recommended to personnel specially instructed and trained in the techniques of real-time PCR.
- Specimens should always be treated as potentially infectious and/or biohazardous material in accordance with safe laboratory procedures.
- 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.
- Discard sample and assay waste according to your local safety regulations.

8 ▸ Workflow

8.1 ▸ Sample Preparation

8.1.1 ▸ Nucleic acid purification

Purified DNA is the sample input material for the ANCHOR HHV-6/7/8 PCR Kit. It has to be ensured that the chosen nucleic acid purification method is compatible with real-time PCR technology. The extraction has to be executed 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 (not more than 3 cycles).

Eluates should be labeled clearly and unambiguously to avoid a mix-up of samples.

8.1.2 ▸ Internal Control

The Internal Control DNA provided with the ANCHOR HHV-6/7/8 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 sample.

In case of manual addition of lysis buffer to the 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 µL of the IC DNA / per reaction should be added to the master mix (see section 8.2.1 Master Mix Set-Up).

8.2 ▸ PCR Preparation

8.2.1 ▸ 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 8.3 PCR Cycler Configuration).

Prepare the Master Mix step by step:

- Thoroughly thaw Master 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 HHV-6/7/8	5 µL (X)	55 µL	Y µL
Master B HHV-6/7/8	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 HHV-6/7/8	5 µL (X)	55 µL	Y µL
Master B HHV-6/7/8	5 µL (X)	55 µL	Y µL
IC DNA	1 µL (X)	11 µL	Y µL
Volume Master Mix	11 µL	121 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 HHV as Positive Control is recommended. For further information, see also chapters 8.4.1 Qualitative Analysis and 8.4.2 Quantitative Analysis, respectively.

Quantitation Standards QS1-4 HHV-6/7/8 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 QS1-4 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.

8.2.2 ▸ PCR Reaction Set-Up

PLEASE NOTE

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 8.2.1 Master Mix Set-Up) into suitable reaction vessels for PCR analysis. This is also valid for Master Mix spiked with IC DNA.
- Add **10 µL of eluate** or control (QS HHV 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

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

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	10 µL	20 µL

8.3 ▸ PCR Cycler Configuration

The ANCHOR HHV-6/7/8 PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 / QuantStudio 5 Dx (Applied Biosystems)	≈ 28 min.
cobas z480 Analyzer (Roche)	≈ 30 min.
LightCycler PRO (Roche)	≈ 30 min.
CFX 96 Deep Well Dx / CFX Opus 96 Dx (Bio-Rad)	≈ 33 / 43 min.
Rotor-Gene Q (QIAGEN)	≈ 44 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.

8.3.1 ▸ General PCR Cycler Settings

Temperature cycling profile for **QuantStudio 5, QuantStudio 5 Dx, LightCycler PRO, cobas z 480 Analyzer, CFX96, CFX Opus 96 and Rotor-Gene Q:**

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

* Fluorescence acquisition for HHV-6/7/8 and IC

Reaction Volume: 20 µL

8.3.2 ▸ Specific PCR Cycler Settings

The following table contains 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 9 Technical Assistance & Contact Information).

QuantStudio 5 / QuantStudio 5 Dx				
Target	HHV-6	IC	HHV-7	HHV-8
Detection	FAM	HEX	TEXAS RED	Cy5.5

Run Settings
- Block Type: 96-Well 0.1-mL Block
- Experiment Type: Standard Curve, Chemistry: TaqMan® Reagents
- Run Mode: Fast
- Plate attributes: Passive Reference - None

Consumables:
- 96-Well Fast Thermal Cycling Plates (Life Technologies Mat. No. 4346907)
- MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971)
- 96-Well-PCR-Plate, Skirted, „Low Profile" white (Starlab Mat. No. E14035209)
- Xtra-Clear Advanced Polyolefin StarSeal (qPCR) (Starlab Mat. No. E2796-9795)

cobas z 480 Analyzer				
Target	HHV-6	IC	HHV-7	HHV-8
Detection	465/510	540/580	610/645	680/700

Run Settings:
- Block size: 96
- 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.

Consumables:
- LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001)
- LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001)
- LC480 Sealing Foil (Roche Mat. No. 04729757001)

LightCycler PRO				
Target	HHV-6	IC	HHV-7	HHV-8
Detection	494/523	541/565	574/601	687/725

Run Settings
- Block size: 96

Consumables:
- LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001)
- LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001)
- LC480 Sealing Foil (Roche Mat. No. 04729757001)

CFX 96 Deep Well Dx / CFX Opus 96 Dx				
Target	HHV-6	IC	HHV-7	HHV-8
Detection	FAM	HEX	TEXAS RED	Cy5.5

Consumables:
- Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655)
- Optical flat 8 Cap Strip for 0.2 mL (Mat. No. TCS0803)
- 0.2 mL 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad Mat. No. TLS 0851)
- 8-strip optical clear flat caps (Sarstedt Mat. No. 65.1998.400)

Rotor-Gene Q				
Target	HHV-6	IC	HHV-7	HHV-8
Detection	Green	Yellow	Orange	Crimson

Run Settings
- Use 72-Well Rotor
- Perform Auto-Gain optimization before 1st acquisition.

Consumables:
- Strip Tubes and Caps, 0.1 mL (QIAGEN Mat. No. 981103)

When preferring a quantitative analysis of the samples, the Quantitation Standards QS1-4 HHV-6/7/8 must be labeled as standards within the instrument software and assigned with their appropriate concentrations.

Quantitation Standard	Concentration [copies/µL]
QS1 HHV-6/7/8	250,000
QS2 HHV-6/7/8	25,000
QS3 HHV-6/7/8	2,500
QS4 HHV-6/7/8	250

8.4 ▸ Data Analysis

The following table contains 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 9 Technical Assistance & Contact Information).

Instrument	Recommendations
QuantStudio 5 / QuantStudio 5 Dx	**Analysis Settings (all channels):** • Plot Type: ΔRn vs Cycle • Graph Type: Linear • Baseline Start/End: 3/15
cobas z 480 Analyzer	**Analysis Settings:** • Abs Quant/2nd Derivative Max • Color Comp (off) • Mean • High Confidence
CFX 96 Deep Well Dx / CFX Opus 96 Dx	**Analysis Settings (all channels):** • Baseline Subtracted Curve Fit • C(t) Determination Mode: Single Threshold • Baseline Threshold: - Baseline Cycles: Auto Calculated
Rotor-Gene Q	**Analysis Settings (all channels):** • Quantitation • Linear Scale • Dynamic Tube ON

8.4.1 ▸ Qualitative Analysis

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

Control	HHV-6	HHV-7	HHV-8	IC
QS3 HHV-6/7/8[1]	+	+	+	+
NC DNA	-	-	-	+

[1] It is recommended to use QS3 HHV-6/7/8 as Positive Control

If one of the conditions has failed, result interpretation of the 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:

Result	HHV-6	HHV-7	HHV-8	IC
HHV-6 DNA positive	+	-	-	+/-
HHV-7 DNA positive	-	+	-	+/-
HHV-8 DNA positive	-	-	+	+/-
HHV-6, -7 and -8 DNA positive	+	+	+	+/-
HHV-6, -7 and -8 DNA negative	-	-	-	+
Invalid	-	-	-	-

A positive result for HHV-6 and/or HHV-7 and/or HHV-8 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 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 HHV-6/7/8 PCR Kit.

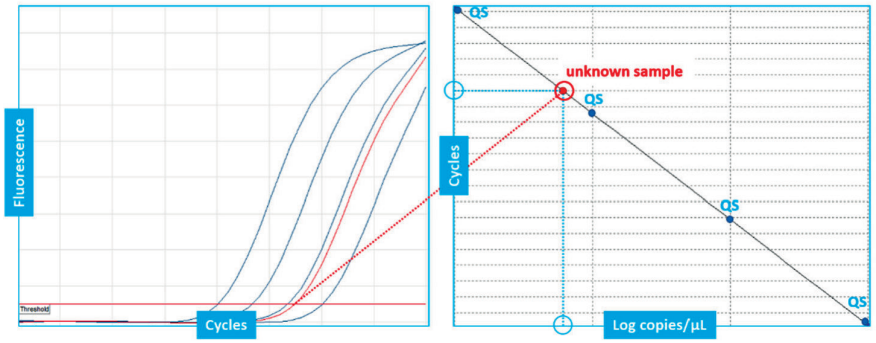
8.4.2 ▸ Quantitative Analysis

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

Control	HHV-6	HHV-7	HHV-8	IC	Correlation Coefficient r^2	Slope m
QS1-4 HHV-6/7/8	+	+	+	+/-[2]	≥0.99	-3.0 to -3.6
NC DNA	-	-	+	-	-	

[2] QS1 and QS2 HHV-6/7/8 are 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 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 HHV-6/7/8 of known concentrations can be used to determine the HHV DNA load present in a sample.



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

$$\textit{Concentration [copies/µL]} = 10^{\frac{C_t - b}{m}}$$

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

The results are displayed as copies/µL. To calculate the concentration of the HHV DNA in the original samples in copies/mL, the concentration factor of the applied sample preparation system must be considered:

$$\textit{Sample}\ \frac{\textit{copies}}{\textit{mL}} = \textit{Eluate}\ \frac{\textit{copies}}{\textit{µL}} \times \frac{\textit{Volume Eluate [µL]}}{\textit{Volume Sample input [mL]}}$$

9 ▸ 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

10 ▶ Symbols

COMP	Component in Kit
CONT	Volume per vial
LOT	Batch code
NUM	Number of vials
QG	Quick Guide - Catalog number and version
REF	Product - Catalog number
RUO	For research use only
UDI	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

Instructions for Use – ANCHOR HHV-6/7/8 PCR Kit

Notes

Notes

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Version 2025-11-19