ANCHOR

HSV 1/2 PCR KIT

Instructions for Use

ANCHOR HSV 1/2 PCR Kit

CE

Quantitative or Qualitative

real-time PCR Kit

for *in vitro* diagnostic use

For *in vitro* diagnostic use

A0900

100

A0910-EN, 2025-10-11

A0911-EN, 2025-06-16

-30°C to -15°C

ANCHOR Diagnostics GmbH
Grandweg 64
D-22529 Hamburg

Instructions for Use – ANCHOR HSV 1/2 PCR Kit

compatible with

QuantStudio 5 (Applied Biosystems)

LightCycler 480 II (Roche)

cobas z 480 Analyzer (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

Mic qPCR (Bio Molecular Systems)

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2 Intended Use

The ANCHOR HSV 1/2 PCR Kit is an *in vitro* nucleic amplification test based on real-time PCR technology for the quantitative or qualitative detection and differentiation of HSV 1 and HSV 2 DNA, isolated from human EDTA plasma, cerebrospinal fluid or cutaneous/mucocutaneous lesions.

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

3 Product Description

The Kit constitutes a ready-to-use system for the amplification, differentiation and quantitation of HSV 1 and HSV 2-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 and differentiation of HSV 1 and HSV 2 specific nucleic acids and the Internal Control in three corresponding detector channels of the real-time PCR instrument.
The Quantitation Standards QS1-4 HSV 1/2 contain defined concentrations of artificial DNA bearing the HSV 1 and HSV 2 target sequences. They 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 HSV 1/2 are used as a whole set, they allow to quantitate HSV 1 and HSV 2 DNA present in a test sample.

4 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 HSV 1 and HSV 2 specific DNA and Internal Control in one reaction setup.
The Quantitation Standards QS1-4 HSV 1/2 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).

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

Kit component	Mat. no.	No. of vials	Quantity	Ingredients
Master A HSV 1/2	A0901	4 vials	4x 125 µL	Buffer, bovine serum albumin, polymerase
Master B HSV 1/2	A0902	4 vials	4x 125 µL	Buffer, salt, nucleotides, target- and IC-specific oligonucleotides
IC DNA	AD00020	1 vial	1000 µL	Buffer, IC-specific synthetic polynucleotide
QS1-4 HSV 1/2*	A0903-1/2/3/4	1 vial each	4x 200 µL	Buffer, target- and IC-specific synthetic polynucleotide
NC DNA*	AD00021	1 vial	200 µL	Buffer, IC-specific synthetic polynucleotide

* INTERNAL CONTROL INSIDE !

5 Storage and Stability

- The ANCHOR HSV 1/2 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 HSV 1/2 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 HSV 1/2, the NC DNA and the IC DNA, thawing and freezing cycles up to 4x are allowed.
- 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 components 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 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 in 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.

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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 HSV 1/2 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.

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9 Workflow
9.1 Sample Preparation
9.1.1 Sample Matrix

The recommended patient sample matrices for sample preparation input are

- human EDTA plasma
- cerebrospinal fluid
- cutaneous and mucocutaneous lesions.

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.

Storage recommendations:
Although several studies reported a relative stability of herpes viruses and their nucleic acids (Hasan et al. & Smit et al.) - if not specified by related manufacturer's instructions - storage of clinical samples under refrigerated conditions (+2°C to +8°C) should not exceed 3-4 days.
For long term storage clinical materials and purified nucleic acids should be frozen (at least -30°C to -15°C).

Short-Term Stability of Pathogen-Specific Nucleic Acid Targets in Clinical Samples
Mohammad R. Hasan, Rusung Tan, Ghada N. Al-Rawahi, Eva Thomas, Peter Tilley
Journal of Clinical Microbiology Nov 2012, 50 (12) 4147-4150

Comparison of collection methods for molecular detection of a-herpes viruses and Treponema pallidum, including evaluation of critical transportation conditions.
Pieter W. Smit, Titia Heijman, Meriem el Abdallaoui, Sylvia M. Bruisten Heliyon 5 (2019) e01522

9.1.2 Sample Preparation

Purified DNA is the sample input material for the ANCHOR HSV 1/2 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.

The diagnostics applicability of the ANCHOR HSV 1/2 PCR Kit has been shown using the following sample preparation platforms:

Sample Preparation Platforms
NucliSENS® easyMag® System (bioMérieux)
EMAG® (bioMérieux)
EZ1 Advanced XL / EZ2 Connect (QIAGEN)
QIAcube Connect (QIAGEN)
QIAsymphony® SP (QIAGEN)
MagNA Pure 96 System (Roche)
MagNA Pure Compact (Roche)
Maxwell® 16 / RSC Instruments (Promega)
KingFisher Systems (Thermo Fisher Scientific)
SEEPREP32™ (Seegene)
GenoXtract® (Hain Lifescience)

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.

9.1.3 Internal Control

The Internal Control DNA provided with the ANCHOR HSV 1/2 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 HSV 1/2	5 µL (X)	55 µL	Y µL
Master B HSV 1/2	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 HSV 1/2	5 µL (X)	55 µL	Y µL
Master B HSV 1/2	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 HSV 1/2 as Positive Control is recommended. For further information, see also chapters 9.4.1 and 9.4.2, respectively.

Quantitation Standards QS1-4 HSV 1/2 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 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.

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9.2.2 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 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 HSV 1/2 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	15 µL	25 µL

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9.3 PCR Cycler Configuration

The ANCHOR HSV 1/2 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.
cobas z 480 Analyzer (Roche)	≈ 30 min.
CFX96 (Bio-Rad)	≈ 33 min.
Rotor-Gene Q (QIAGEN)	≈ 44 min.
Mic qPCR (Bio Molecular Systems)	≈ 34 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 General PCR Cycler Settings

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

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

* Fluorescence acquisition for HSV 1, HSV 2 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 HSV 1, HSV 2 and IC

Reaction volume: 25 µL

9.3.2 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 12 Technical Assistance & Contact Information).

QuantStudio™ 5			
Target	HSV 1	IC	HSV 2
Detection	FAM	HEX	TEXAS RED

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)

LightCycler® 480 II (cobas z 480 Analyzer)			
Target	HSV 1	IC	HSV 2
Detection	465/510	533/580 (540/580)	618/660 (610/645)

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)

Bio-Rad CFX96			
Target	HSV 1	IC	HSV 2
Detection	FAM	HEX	TEXAS RED

Consumables:
- Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655)
- Optical flat 8 Cap Strip for 0.2mL (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	HSV 1	IC	HSV 2
Detection	Green	Yellow	Orange

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)

Mic qPCR			
Target	HSV 1	IC	HSV 2
Detection	Green	Yellow	Orange

Run Settings
- Temperature Control: Standard TAQ

Consumables:
- Mic Tubes and Caps (Mat. No. 68MIC-60653)

When preferring a quantitative analysis of the clinical samples, the Quantitation Standards QS1-4 HSV 1/2 must be labeled as standards within the instrument software and assigned with their appropriate concentrations.

Quantitation Standard	Concentration [cop/µL]
QS1 HSV 1/2	50,000
QS2 HSV 1/2	5,000
QS3 HSV 1/2	500
QS4 HSV 1/2	50

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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):
- Plot Type: ΔRn vs Cycle
- Graph Type: Linear
- Baseline Start/End: 3/15
- Threshold:
  - FAM 200,000
  - HEX 75,000
  - TEXAS RED: 200,000

LightCycler® 480 II (cobas z 480 Analyzer)

Analysis Settings:
- Abs Quant/2nd Derivative Max
- Color Comp (off)
- Mean
- High Confidence
  - 465/510: 0.6
  - 533/580: 2.2
  - 618/660: 0.1

Bio-Rad CFX96

Analysis Settings (all channels):
- Baseline Subtracted Curve Fit
- C(t) Determination Mode: Single Threshold
- Baseline Threshold:
  - Baseline Cycles: Auto Calculated
  - Single Threshold
    - FAM: 900
    - HEX: 500
    - TEXAS RED: 900

Rotor-Gene Q
Analysis Settings (all channels): ▪ Quantitation ▪ Linear Scale ▪ Dynamic Tube ON ▪ Threshold: - Green: 0.08 - Yellow: 0.08 - Orange: 0.10

Mic qPCR
Analysis Settings (all channels): ▪ Graph Type: Linear ▪ Method: Dynamic ▪ Ignore Cycles Before: 3 ▪ Threshold Start: 1 ▪ Exclusion: None ▪ Threshold Level: - Green: 1.50 - Yellow: 1.50 - Orange: 1.50

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	HSV 1	HSV 2	IC
QS 3 HSV 1/2 [1]	+	+	+
NC DNA	-	-	+

[1] It is recommended to use QS3 HSV 1/2 as Positive Control, but the criteria also apply to QS4 HSV 1/2. QS1-2 HSV 1/2 are excluded from these criteria. The presence of high concentrated artificial nucleic acids in those Quantitation Standards can result in a competitive inhibition of the IC amplification.

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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	HSV 1	HSV 2	IC
HSV 1 and HSV 2 DNA positive	+	+	+/-
HSV 1 DNA positive	+	-	+/-
HSV 2 DNA positive	-	+	+/-
HSV 1/2 DNA negative	-	-	+
Invalid	-	-	-

A positive result for HSV 1 and/or HSV 2 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 HSV 1/2 PCR Kit.

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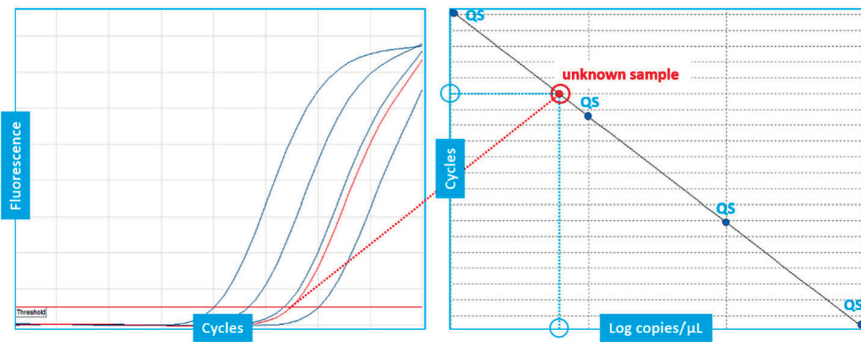
9.4.2 ▸ Quantitative Analysis

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	HSV 1	HSV 2	IC	Correlation Coefficient r^2	Slope m
QS1-4 HSV 1/2	+	+	(+)[2]	≥ 0.99	-3.0 to -3.6
NC DNA	-	-	+	-	-

[2] QS1 and QS2 HSV 1/2 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 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 HSV 1/2 of known concentrations can be used to determine the HSV 1 and HSV 2 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 copies/µL. To calculate the concentration of HSV 1 and HSV 2 DNA in the original clinical sample in copies/mL, the concentration factor of the applied sample preparation system must be considered:

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

10 ▸ Performance Data
10.1 ▸ Analytical Performance
10.1.1 ▸ Sensitivity

The LOD for the ANCHOR HSV 1/2 PCR Kit was determined by undertaking a probit analysis on the Rotor-Gene Q platform. A dilution series of different concentration levels for HSV 1 DNA (ATCC / LGC Mat # VR-1493D) and HSV 2 DNA (ATCC / LGC Mat # VR-734D) 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 5 instruments.

Instrument	HSV 1 LOD	HSV 2 LOD	Unit
QuantStudio 5	0.24	0.16	copies/µL
LightCycler 480 II (Roche)	0.16	0.08	copies/µL
cobas z 480 Analyzer	0.08	0.08	copies/µL
CFX96	0.16	0.16	copies/µL
Rotor-Gene Q	0.08	0.08	copies/µL
Mic qPCR	0.08	0.16	copies/µL

10.1.2 ▸ Linearity

The linear range of the ANCHOR HSV 1/2 PCR Kit was determined by testing a dilution series of artificial DNA comprising the HSV 1 or the HSV 2 PCR target, ranging from 1.00E+07 copies/µL to 1.00E+00 copies/µL. The dilutions were analyzed 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 HSV 1/2 PCR Kit lots.

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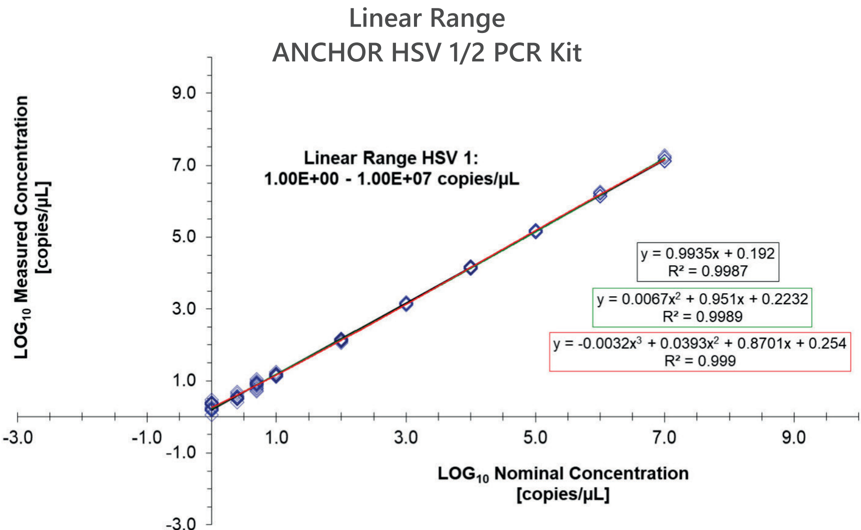

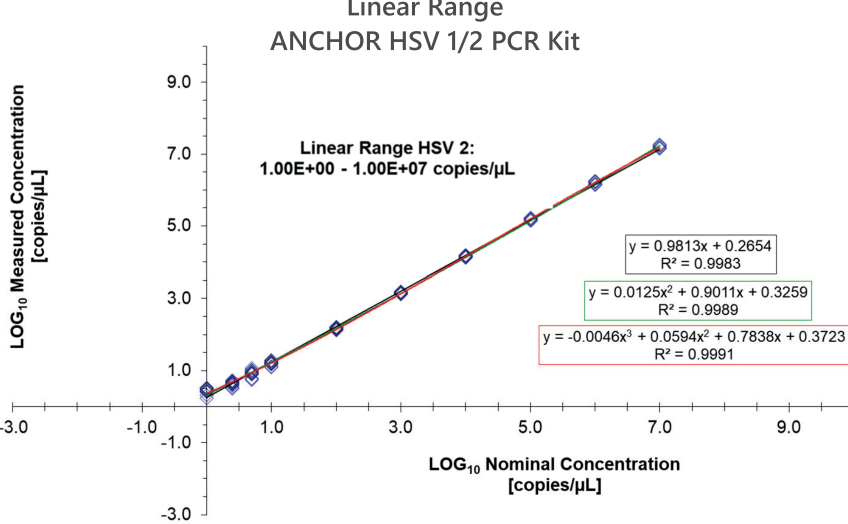

The linear quantification range for the ANCHOR HSV 1/2 PCR Kit was tested and confirmed to be from at least 1.00E+00 up to 1.00E+07 copies/µL for both HSV 1 and HSV 2 DNA. The Limit of Quantitation (LOQ) for HSV 1 and HSV 2 is therefore determined to be 1.00E+00 copy/µL.

10.1.3 Specificity

Triplicates of three different HSV 1 and HSV 2 strains were tested at a concentration near the 3x LOD of the ANCHOR HSV 1/2 PCR Kit.

Strain	HSV 1	HSV 2
HSV 1 strain MacIntyre	+	-
HSV 1 strain KOS	+	-
HSV 1 type 95	+	-
HSV 2 strain MS	-	+
HSV 2 strain G	-	+
HSV 2 type 09	-	+

Nucleic acid of selected pathogens with a concentration of ≈5.00E+03 copies/µL (alternative units CFU/µl or TCID50/µL) was added to the PCR reaction and tested in triplicates in the absence or presence of HSV 1 and HSV 2 DNA at their 3x LOD and 3x LOQ concentrations on the Rotor-Gene Q.

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Pathogen	- HSV 1/2		3x LOD HSV 1/2		3x LOQ HSV 1/2 Δ Log_{10} cop/µL[3]	
	HSV 1	HSV 2	HSV 1	HSV 2	HSV 1	HSV 2
VZV	-	-	+	+	-0.02	-0.16
BK Virus	-	-	+	+	-0.08	-0.16
Human Cytomegalovirus	-	-	+	+	0.01	-0.09
Epstein-Barr Virus	-	-	+	+	0.01	0.04
Hepatitis B Virus	-	-	+	+	0.04	-0.09
Human Herpesvirus 7	-	-	+	+	-0.08	-0.14
Human Parvovirus B19	-	-	+	+	0.09	0.03
Enterovirus 71	-	-	+	+	-0.04	-0.14
West-Nile Virus	-	-	+	+	-0.10	-0.13
Candida albicans	-	-	+	+	-0.02	-0.02
Neisseria gonorrhoeae	-	-	+	+	-0.03	-0.12
Gardnerella vaginalis	-	-	+	+	-0.06	-0.16
Clostridium perfringens	-	-	+	+	-0.07	-0.09
Escherichia coli	-	-	+	+	0.00	-0.09
Salmonella typhimurium	-	-	+	+	-0.07	-0.10

[3] In relation to HSV 1 and HSV 2 DNA only control

Limitations:

- HSV 1:
  - At concentrations near the detection limit, potentially co-infecting HSV 2 at a concentration of >50 copies/µL will interfere with HSV 1 detectability.
  - At concentrations near the quantitation limit, potentially co-infecting HSV 2 at a concentration of >500 copies/µL will interfere with reliable HSV 1 quantitation.
- HSV 2:
  - At concentrations near the detection limit, potentially co-infecting HSV 1 at a concentration of >50 copies/µL will interfere with HSV 2 detectability.
  - At concentrations near the quantitation limit, potentially co-infecting HSV 1 at a concentration of >500 copies/µL will interfere with reliable HSV 2 quantitation.

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 HSV 1		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	19.13	0.04	0.21
Inter-Run	19.38	0.19	0.99
Inter-Batch	19.26	0.09	0.47
Total	19.33	0.18	0.92
	QS4 HSV 1		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	29.07	0.17	0.57
Inter-Run	29.28	0.27	0.94
Inter-Batch	29.08	0.17	0.60
Total	29.21	0.26	0.90
	QS HSV 1 LOQ (1 copy/µL)		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	35.49	0.53	1.50
Inter-Run	34.84	0.60	1.71
Inter-Batch	34.80	0.63	1.81
Total	34.86	0.63	1.80
	ATCC HSV 1 LOQ (1 copy/µL)		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	34.21	0.45	1.32
Inter-Run	33.37	0.61	1.83
Inter-Batch	33.82	0.45	1.34
Total	33.56	0.62	1.84

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	QS1 HSV 2		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	19.20	0.07	0.38
Inter-Run	19.14	0.24	1.26
Inter-Batch	19.13	0.22	1.13
Total	19.12	0.24	1.24
	QS4 HSV 2		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	29.24	0.13	0.45
Inter-Run	28.96	0.26	0.91
Inter-Batch	28.86	0.22	0.78
Total	28.91	0.26	0.91
	QS HSV 2 LOQ (1 copy/µL)		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	34.54	0.52	1.50
Inter-Run	34.33	0.48	1.39
Inter-Batch	34.07	0.69	2.02
Total	34.24	0.61	1.78
	ATCC HSV 2 LOQ (1 copy/µL)		
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	33.45	0.46	1.36
Inter-Run	33.10	0.62	1.87
Inter-Batch	33.54	0.35	1.03
Total	33.24	0.58	1.75

Precision of the ANCHOR HSV 1/2 PCR Kit in combination with the other instruments was evaluated for intra- and inter-run variability.

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10.2. Clinical Performance

The clinical performance of the ANCHOR HSV 1/2 PCR Kit for the qualitative and quantitative detection and differentiation of HSV 1 and HSV 2 DNA in human CSF samples and cutaneous/mucocutaneous swabs was evaluated comparatively at 3 different study sites against established HSV 1/2 routine diagnostic workflows using CE-marked PCR assays as reference standard.

581 prospectively collected specimen were analyzed with the ANCHOR HSV 1/2 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 LightCycler 480 II and QuantStudio 5.

		Comparators	
	∑ 581	POS	NEG
ANCHOR HSV 1/2 PCR Kit	POS	148	10
	NEG	4	419

PPA: 97.4 % NPA: 97.7 %

The clinical performance for human EDTA plasma was evaluated at 2 different study sites against an established HSV 1/2 routine diagnostic workflow using a CE-marked PCR assay as reference standard.
213 EDTA plasma samples were analyzed with the ANCHOR HSV 1/2 PCR Kit and with the comparator assay to determine their positive percent agreement (PPA) and negative percent agreement (NPA), respectively. Testing was done using the Mic qPCR cycler.

	HSV 1	Comparator	
	∑ 213	POS	NEG
ANCHOR HSV 1/2 PCR Kit	POS	51	10
	NEG	0	152

PPA: 100 % NPA: 93.8 %

	HSV 2	Comparator	
	∑ 213	POS	NEG
ANCHOR HSV 1/2 PCR Kit	POS	30	2
	NEG	0	181

PPA: 100 % NPA: 98.9 %

11 Quality Control

In accordance with the implemented ISO 13485-certified Quality Management System, each lot of the ANCHOR HSV 1/2 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

13 Symbols

COMP Component in Kit

CONT Volume per vial

IVD For *in vitro* diagnostic use

LOT Batch code

NUM Number of vials

QG Quick Guide - Catalog number and version

REF Product - Catalog number

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

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