# 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



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#### compatible with

LightCycler 480 II (Roche)

cobas z 480 Analyzer (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

QuantStudio 5 (Applied Biosystems)

Mic qPCR (Biomolecular 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 2 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 2 are supplied with the IC (Internal Control) DNA 2 already incorporated (see also section 9.2.1 Master Mix Set-Up).

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

Master A HSV 1/2	Master B HSV 1/2	IC DNA 2	! QS1-4 HSV 1/2	NC NA 2
A0901	A0902	A0022	A0903- 1/2/3/4	A0032
4 Vials	4 Vials	1 Vial	1 Vial each	1 Vial
4x 125 μL	4x 125µL	1000 μL	4x 200 μL	200 µL
Contains: Buffer, Bovine Serum Albumin, Poly- merase	Contains: Buffer, Salt, Nucleotides, Target- and IC-specific Oli- gonucleotides	Contains: Buffer, IC-spe- cific synthetic Polynucleotide	Contains: Buffer substan- ce, Target-spe- cific synthetic Polynucleotide	Contains: Buffer substance, 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 2 and the IC DNA 2, 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 diagnostic results 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.
- 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 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 ED-TA-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 more than 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 systems:

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



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 labelled clearly and unambiguously to avoid a mixup of samples.

#### 9.1.3 Internal Control

The Internal Control DNA 2 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.



The Internal Control DNA 2 MUST NOT be added directly to the clinical sample.

Always add the Internal Control DNA 2 after lysis buffer has been added to the sample.

The required volume of Internal Control DNA 2 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 2 volume: 20 μL
- Elution buffer per sample: 60  $\mu$ L -> IC DNA 2 volume: 6  $\mu$ L



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 2 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  $\mu$ L of the IC DNA 2 / per reaction should be added to the master mix (see section 9.2.1 Master Mix Set-Up).

#### 9.2 PCR Preparation

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#### 9.2.1 Master Mix Set-Up

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 2 present in sample eluates – NO IC DNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A HSV 1/2	5 μL (X)	55 µL	ΥμL
Master B HSV 1/2	5 μL (X)	55 µL	ΥμL
Volume Master Mix	10 μL	110 μL	ZμL

\*10 reactions + 10%

\*\* See formula on next page

**IC DNA 2 to be used as inhibition control only –** IC DNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A HSV 1/2	5 μL (X)	55 μL	ΥμL
Master B HSV 1/2	5 μL (X)	55 μL	ΥμL
IC DNA 2	1.5 μL (X)	16.5 μL	ΥμL
Volume Master Mix	11.5 μL	126.5 μL	ZμL

\*10 reactions + 10%

\*\* See formula on next page



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*×*X*µ*Lx*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 2 already contain the IC DNA 2 in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC DNA 2 (as inhibition control) in combination with the QS1-4 and NC DNA 2, 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



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 2) 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  $\mu$ L 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 2.
- Add 15 μL of eluate or control (Quantitation Standards QS1-4 HSV 1/2 or Negative Control DNA 2). 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!



Carefully handle reaction vessels during transfer to avoid mix up of 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 to achieve an optimum amplification curve performance !!!

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

#### 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 (BMS)	≈ 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:** 

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

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

#### Temperature cycling profile for Mic qPCR:

	95°C	1 sec	
Cycling	63°C *	2 sec	x 40
	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, colour compensation, gain optimisation settings, etc. do not hesitate to contact us directly (see section 12 Technical Assistance & Contact Information).

Instrument	Target	Detection channel	Recommendations / Requirements
	HSV 1	465/510	Run Settings: • Block size: 96 • If clear plates are used, the sensor of the LightCycler <sup>®</sup> has to be disabled
LightCycler™ 480 II (Cobas z 480 Analyzer)	IC	533/580 (540/580)	by selecting the Clear Plates option in the software before the run is started. <b>Consumables:</b> • LC480 Multiwell Plate 96, white Roche Mat. No. 04729692001)
	HSV 2	618/660 (610/645)	<ul> <li>LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001)</li> <li>LC480 Sealing Foil (Roche Mat. No. 04729757001)</li> </ul>
	HSV 1	FAM	Consumables: • Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655) • Optical flat 8 Cap Strip for
Bio-Rad CFX96	IC	HEX	0.2ml (Mat. No. TCS0803) • 0.2 ml 8-Tube PCR Strips without Caps, low profile, white
	HSV 2	TEXAS RED	(Bio-Rad Mat. No. TLS 0851) • 8-strip optical clear flat caps (Sarstedt Mat. No.65.1998.400)

Instrument	Target	Detection channel	Recommendations / Requirements
	HSV 1	Green	Run Settings: • Use 72-Well Rotor • Perform Auto-Gain optimisation before
Rotor-Gene Q	IC	Yellow	1 st acquisition. Consumables:
	HSV 2	Orange	<ul> <li>Strip Tubes and Caps, 0.1 ml (QIAGEN Mat. No 981103)</li> </ul>
	HSV 1	FAM	Run Settings: • Block Type: 96-Well 0.1-mL Block • Experiment Type: Standard Curve Chemistry: TaqMan® Reagents
QuantStudio™ 5	IC	HEX	<ul> <li>Run Mode: Fast</li> <li>Plate attributes:</li> <li>Passive Reference - None</li> </ul>
	HSV 2	TEXAS RED	Consumables: • 96-Well Fast Thermal Cycling Plates (Life Technologies Mat.No. 4346907) • MicroAmp <sup>™</sup> Optical Adhesive Film (Life Technologies Mat. No. 4311971)
	HSV 1	Green	Run Settings: • Temperature Control: Standard TAQ
Mic qPCR	IC	Yellow	Mic Tubes and Caps (Mat. No.68MIC-60653)
	HSV 2	Orange	

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

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

#### 9.4 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 12 Technical Assistance & Contact Information).

Instrument	Recommendations <sup>1</sup>
LightCycler <sup>®</sup> 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 Substracted 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
QuantStudio™ 5	Analysis Settings (all channels): • Plot Type: ∆Rn vs Cycle • Graph Type: Linear • Baseline Start/End: 3/15 • Threshold: - FAM 200,000 - HEX 75,000 - TEXAS RED: 200,000
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

<sup>1</sup> Cycler- or run file-specific threshold settings might be necessary



#### 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
QS3 HSV 1/2 <sup>2</sup>	+	+	+
NC DNA 2	-	-	+

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.

<sup>&</sup>lt;sup>2</sup> 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.

#### 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
QS1-2 HSV 1/2	+	+	+/-
QS3-4 HSV 1/2	+	+	+
NC DNA 2	-	-	+
Channel/Target	Correlation Coefficient r <sup>2</sup>		Slope m
QS1-4 HSV 1/2	≥ 0.99		-3.0 to -3.6
NC DNA 2	-	-	

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/  $\mu$ L. 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 was then confirmed or re-evaluated on the other 5 instruments.

Instrument	HSV 1 LOD	HSV 2 LOD	Unit
Rotor-Gene Q	0.08	0.08	copies/µL
QuantStudio 5	0.24	0.16	copies/µL
LightCycler 480II	0.16	0.08	copies/µL
CFX96	0.16	0.16	copies/µL
Cobas z 480	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/ $\mu$ L to 1.00E+00 copies/ $\mu$ L. 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 HSV 1/2 PCR Kit lots. The picture below shows scatter charts for HSV 1 and HSV 2 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 HSV 1/2 PCR Kit was tested and confirmed to be from at least 1.00E+00 up to 1.00E+07 copies/ $\mu$ L 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/ $\mu$ L.

#### 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  $\approx$ 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.

Pathogen	- HS	V 1/2	3x LO 1	D HSV /2	$3$ x LOO $\Delta$ Log <sub>10</sub>	HSV 1/2 copies/µL <sup>3</sup>
	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

 $^{\rm s}$  In relation to HSV 1 and HSV 2 DNA only control

Limitations:

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

#### 10.1.4 Precision

Precision testing was initially performed on the Rotor-Gene Q instrument. For intra-run variability, 3-6 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 F	ISV 1 LOQ (1 cop	y/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 coj	oy/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

		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 H	ISV 2 LOQ (1 cop	y/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 coj	py/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.

#### 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 analysed 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 480II and QuantStudio 5.

		Comparators	
	∑ 581	POS	NEG
Anchor	POS	148	10
HSV 1/2 PCR Kit	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 analysed 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	POS	51	10
HSV 1/2 PCR Kit	NEG	0	152

PPA: 100 % NPA: 93.8 %

	HSV-2	Comparator	
	∑ 213	POS	NEG
Anchor	POS	30	2
HSV 1/2 PCR Kit	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 40 520 14 830 email: support@anchor-diagnostics.com

#### **Symbols** 13

- IVD
- For in vitro diagnostic use
- REF Product Catalogue number
- Σ
- Contains sufficient reagents for <N> tests
- HB
  - Instructions for Use Catalogue number and version



Consult Instructions for Use



Quick Guide - Catalogue number and version



Temperature limits for storage



Use by



Batch code



Important Note



Manufacturer



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