Instructions for Use
Life Science Kits & Assays

RoboGene® HSV DNA Qualitative Kit

analytikjena
An Endress+Hauser Company

Order No.:
For use with Rotor-Gene® 3000/6000/Q (0.2 ml)
847-0207500642 116 reactions
847-0207500644 - 58 reactions
For use with MiniOpticon, CFX and 7500 Fast/LP (0.1 ml)
847-0207500662 120 reactions
847-0207500664 64 reactions
For use with ABI PRISM® 7000/7300 SDS
847-0207500602 120 reactions
847-0207500604 64 reactions
For use with LightCycler® 480 II, Toptical and qTOWER 2&3/LPW (0.1 ml)
847-0207500682 120 reactions
847-0207500684 64 reactions

Publication No.: Manual_RoboGene_HSV_DNA_Qualititive_Kit_e_rev2

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1 Introduction

1.1 Intended use

The RoboGene® HSV DNA Qualitative Kit is intended for real-time detection of *Herpes simplex* Virus 1 and 2 (*HSV-1* and *HSV-2*) DNA in human serum, EDTA blood, liquor, blister aspirates, tissue biopsies, and swabs of lesions, rashes or ulcer samples. The kit is available for application to Rotor-Gene® 3000/6000/Q, MiniOpticon, CFX96, ABI® 7500 Fast, ABI PRISM® 7000/7300 SDS, TOptical, qTOWER 2&3 or LightCycler® 480 II.

The RoboGene® HSV DNA Qualitative Kit is intended for research use only but not for diagnostic procedures.

1.2 Pathogen information

Herpes Simplex Virus type 1 and type 2 (*HSV-1* and *HSV-2*) may cause a variety of clinical symptoms such as mucocutaneous lesions and infections of the central nervous system (1-4). They are also called *Human Herpes Virus* 1 and 2 (*HHV-1* and *HHV-2*) and are neurotropic and neuroinvasive viruses. They enter and hide in the human nervous system, accounting for their durability in the human body. *HSV-1* is commonly associated with herpes outbreaks of the face known as cold sores or fever blisters, whereas *HSV-2* is more often associated with genital herpes. Neonatal infection following exposure to the virus at delivery can produce severe disseminated infection and death. In immunocompromised patients, the virus leads to severe clinical outcomes, including mucocutaneous disease and pneumonia (2).

CONSULT INSTRUCTION FOR USE

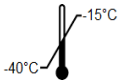
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.3 Technical assistance

In case of any problem with the RoboGene® HSV DNA Qualitative Kit please contact our technical support team which consists of experienced scientists with long-time experience in the field of molecular diagnosis particularly of real-time PCR detection of pathogens. For technical assistance please contact us as shown inside the cover of the IFU.

1.4 Notes on the use of this instructions for use

For easy reference and orientation, the IFU uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number
 N	**Content** Contains sufficient reagents for <N> tests
	**Storage conditions**
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Use by**
	**Lot number** Lot number of the kit or component
	**Manufactured by**
COMP	**Component**
VOL	**Volume**
	Note / Attention Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following abbreviations are used in the IFU:

Ct	Threshold cycle value
dTTP	2′-deoxythymidine 5′-triphosphate
dUTP	2′-deoxyuracyl 5′-triphosphate
dNTP	2′-deoxynucleotide 5′-triphosphate
HSV	*Herpes Simplex Virus*
IC	Internal Control
IFU	Instruction For Use
IU	International Units
NTC	Non-template control
PEI	Paul-Ehrlich-Institut, Langen, Germany
SD	Standard deviation

2 Safety precautions

NOTE
Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the IFU, as well as all messages and information, which are shown.

- Human plasma or serum samples have to be considered as potentially infectious. Thus, always wear lab coat and gloves.
- Always use clean and nuclease-free equipment.
- Set up of template preparation, PCR reagent assembly, amplification and detection should be performed in different rooms.
- Discard sample and assay waste according to your in-house safety regulations.

ATTENTION
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

3 Test description and principle

3.1 Principle of the TaqMan® assay

TaqMan® real-time PCR is a highly sensitive assay that combines amplification with fluorescence-based online detection of the nucleic acid of interest (target, template). The assay is based on a conventional set of target-specific primers in combination with a fluorescence-labelled oligonucleotide probe, complementary to the desired target sequence. In the presence of target the probe hybridizes with its target-complementary sequence. The Taq DNA polymerase possesses a 5`→ 3` exonuclease activity that cleaves the probe and displaces the fluorescent dye from the quencher. This event results in an increase of the fluorescence signal, which is directly proportional to the target amplification during each PCR cycle.

3.2 Explanation of the HSV Qualitative test

The RoboGene® HSV DNA Qualitative Kit is an amplification test for detection of *HSV* DNA in human liquor, blister aspirates, tissue and swab samples. All virus types (*HSV-1* and *HSV-2*) are amplified with equal efficiency applying probes and primers specific for a subsequence of the HSV genome encoding parts of the DNA polymerase (UL 30) gene. The qualitative standard consists of a set of 4 tubes or stripes coated with known amounts of synthetic *HSV-1* or *HSV-2* DNA, respectively, which must be amplified in parallel (Table 15).

A synthetic internal control is included via extraction tubes to control DNA extraction and to indicate for inhibitory effect on detection. Thus, the risk concerning false-negative results is drastically reduced yielding in an increase of detection correctness. Amplification of *HSV* DNA in samples and standards and of IC DNA is measured independently at different wavelengths due to probes labelling with different fluorescence reporter dyes (HSV DNA: FAM channel; IC DNA: Yakima Yellow/VIC/JOE channel).

For sample preparation the “INSTANT Virus RNA/DNA Kit” (AJ Innuscreen) is recommended. DNA extraction must be performed with the respective starting sample volume strictly according to manufacturer’s instructions. Concerning final elution of filter bound DNA the use of 60 µl of elution buffer is supposed, respectively.

NOTE
The respective Internal Control is stabilized within the extraction tubes contained in the RoboGene® HSV DNA Qualitative Kit!

3.3 Restrictions

This test is validated for use together with human EDTA plasma or serum. Heparinized and lipaemic plasma or serum has to be excluded from analysis. If other than the recommended sample types are used wrong results may be obtained. The product is to be used by personnel specially instructed and trained in a laboratory environment. Strict compliance with the instruction for use is required for optimal PCR results. Do not use expired components or mix with components from different batches.

4 Performance assessment

4.1 Analytical sensitivity

The analytical sensitivity is defined as the smallest amount of target that can be precisely detected in a sample. Using synthetic *HSV* DNA in different dilutions a >95 % cut-off value of at least 10 copies was calculated for the assay. Individual values below the detection limit may be plausible but with a high probability of error. To reduce this error probability 3 replicates of such samples are recommended.

4.2 Linear range

The linear range of the RoboGene® HSV DNA Qualitative Kit was found from 10 to at least 1x 10^9 copies per run as shown in Figure 2. This evaluation was performed with serial dilutions of a synthetic HSV-1 DNA and HSV-2 DNA specimen and 3 replicates at each level. Each dilution was tested on Rotor-Gene® 3000.

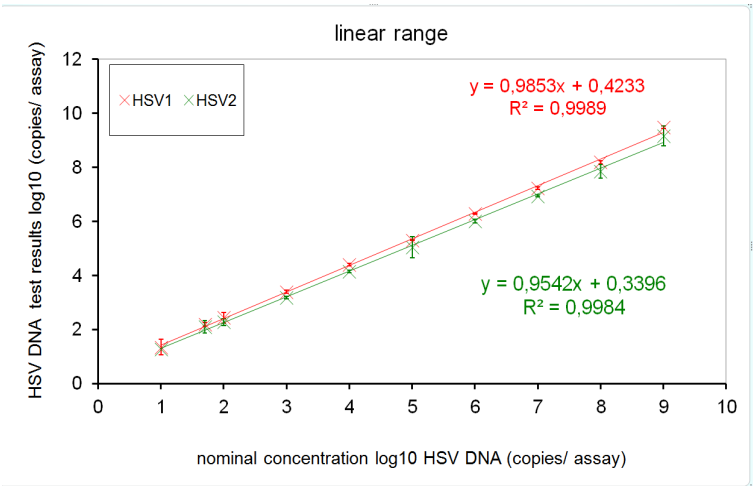


Figure 1: Linearity of the RoboGene® HSV DNA Qualitative Kit on Rotor-Gene® 3000 using synthetic, HPLC calibrated HSV DNA specimen

4.3 Specificity

4.3.1 HSV genotype testing

Two *HSV* genotypes (1 and 2) have been defined based on nucleotide divergence within the complete genome (1-4). The performance of the RoboGene® HSV DNA Qualitative Kit on *HSV* genotypes was evaluated with the HSV reference strains Kupka (*HSV* Type 1, proliferated in Vero cells, 108.1 TCID50/ml) and US (*HSV* Type 2, proliferated in Vero cells, 107.1 TCID50/ml), kindly provided by Prof. Dr. Sauerbrei, University Hospital Jena, Institute of Virology & Antiviral Treatment, German Reference Lab for *HSV* infections. The study demonstrated that the *HSV* genotypes 1 and 2 are amplified with similar efficiency (Figure 2).

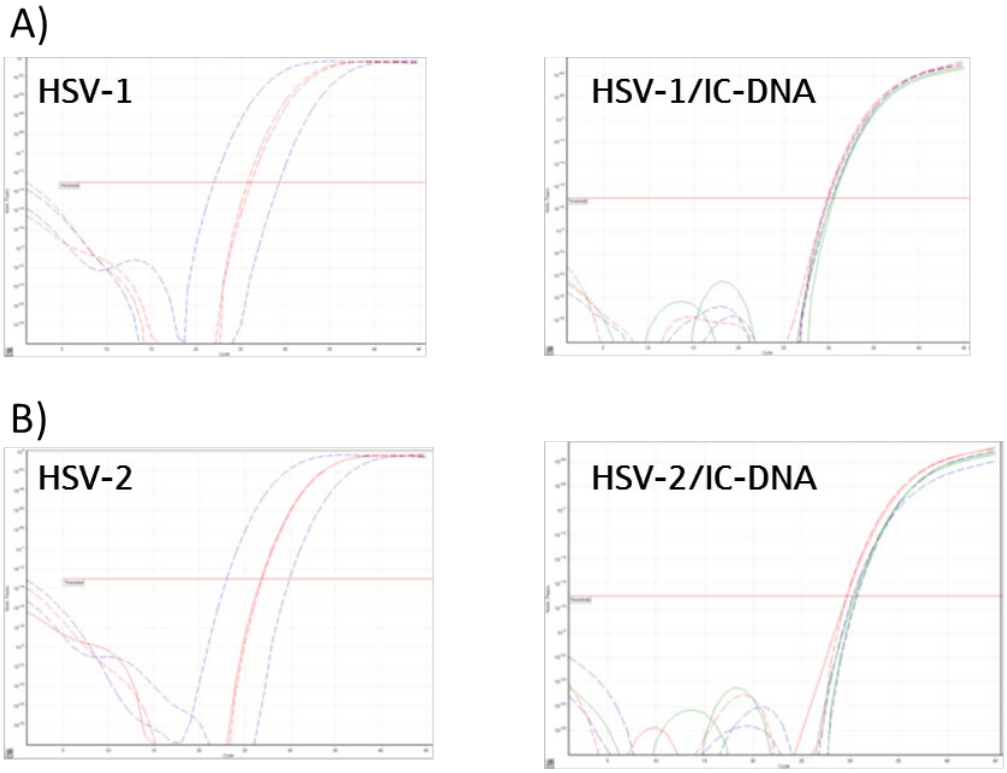


Figure 2: *HSV* reference strain analysis. A) Strain Kupka (*HSV* Type 1, proliferated in Vero cells, 108.1 TCID50/ml), diluted 1:1000 (red) before DNA purification. B) Strain US (*HSV* Type 2, proliferated in Vero cells, 107.1 TCID50/ml), diluted 1:1000 (red) before DNA purification. Right figures: corresponding IC-DNA amplification curves. Purple lines: qualitative standards, 10,000 and 100 copies per run, respectively and green lines *HSV* negative controls.

4.3.2 Analytical specificity

HSV negative samples were analyzed to determine the specificity of the RoboGene® HSV DNA Qualitative Kit, which is expressed as negative result in absence of the target. The analytical specificity was evaluated by analyzing 18 non-*HSV* positive specimens (Table 1). No *HSV* DNA (FAM) signal should be detected in these specimens.

Table 1: Pathogen samples used for analysis of analytical specificity

Control group	HSV (FAM)	IC DNA (JOE/ VIC)
DNA virus		
Hepatitis B virus (HBV), n=5	0/5	5/5
Human Cytomegalovirus (HCMV), n=5	0/5	5/5
Parvovirus B19 (PVB19), n=1	0/1	1/1
RNA virus		
Hepatitis A virus (HAV), n=1	0/1	1/1
Hepatitis C virus (HCV), n=4	0/4	4/4
Hepatitis D virus (HDV), n=1	0/1	1/1
Human immunodeficiency virus 1 (HIV-1), n=1	0/1	1/1

Furthermore, 15 plasma samples from blood donors which have been tested negative for *HSV* DNA were analyzed (Table 2).

Table 2: Specificity of the RoboGene® HSV DNA Qualification Kit

Analysed samples	HSV positive	IC-DNA positive
HSV negative plasma (n=15)	0	15

The RoboGene® HSV DNA Qualitative Kit had a perfect analytical specificity. None of the analyzed samples gave positive test results for *HSV* DNA.

5 Kit components, storage and stability

Table 3: General kit components of RoboGene® HSV DNA Qualitative Kit

General kit components				
Component	Σ 58/64	Σ 116/120	Description	Box No.
DNA_D1	50 tubes	100 tubes	Extraction tubes coated with IC DNA and carrier nucleic acid	2
HSV_D4 	2 vials	4 vials	Reagent mix lyophilized with HSV/IC-specific primers, -probes and dNTPs	1
Taq Polymerase FS1 	1 x 0.03 ml	2 x 0.03 ml	Taq Polymerase, 5 U/µl	separate
10x PCR buffer FS1 	1 x 0.50 ml	1 x 0.50 ml	10x PCR buffer with $MgCl_2$	separate
PCR grade water DNA 	2 x 1.50 ml	4 x 1.50 ml	PCR grade water	1
IFU	1	1		1

Table 4: Kit components for application to Rotor-Gene® 3000/6000/Q using regular profile tubes 0.2 ml (clear)

Component	Σ 58	Σ 116	Description	Box No.
REF	847-0207500644	847-0207500642		
HSV_D2_RG	50 tubes	100 tubes	Sample tubes coated with amplification enhancer	1
HSV_D3_RG	2 strips (2x 4 tubes)	4 strips (4x 4 tubes)	Qualitative standard tubes coated with 2 different amounts of *HSV*-1 and *HSV*-2 DNA, respectively, IC DNA and amplification enhancer	1

Table 5: Kit components for application to TOptical, qTOWER2&3 and LightCycler® 480 II using low profile strips 0.1 ml (white)

Component	Σ 64	Σ 120	Description	Box No.
REF	847-0207500684	847-0207500682		
HSV_D2_LPW	7 strips (7x 8 tubes)	13 strips (13x 8 tubes)	Sample tubes coated with amplification enhancer	1
HSV_D3_LPW	2 strips (2x 4 tubes)	4 strips (4x 4 tubes)	Qualitative standard tubes coated with 2 different amounts of *HSV*-1 and *HSV*-2 DNA, respectively, IC DNA and amplification enhancer	1
OT_AB	1	2	Optical tape	1

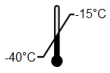
Table 6: Kit components for application to MiniOpticon, CFX96 and ABI® 7500 Fast using low profile strips 0.1 ml

Component	Σ 64	Σ 120	Description	Box No.
REF	847-0207500664	847-0207500662		
HSV_D2_LP	7 strips (7x 8 tubes)	13 strips (13x8 tubes)	Sample tubes coated with amplification enhancer	1
HSV_D3_LP	2 strips (2x 4 tubes)	4 strips (4x 4 tubes)	Qualitative standard tubes coated with 2 different amounts of *HSV*-1 and *HSV*-2 DNA, respectively, IC DNA and amplification enhancer	1
OT_AB	1	2	Optical tape	1
10x ROX [1)]	1x 0.50 ml	1x 0.50 ml	10x ROX passive reference dye	1

[1)] Kit component not required for use with MiniOpticon and CFX96

Table 7: Kit components for application to ABI PRISM® 7000/7300 SDS

Component	Σ 64	Σ 120	Description	Box No.
REF	847-0207500604	847-0207500602		
HSV_D2_AB	7 strips (7x 8 tubes)	13 strips (13x 8 tubes)	Sample tubes coated with amplification enhancer	1
HSV_D3_AB	2 strips (2x 4 tubes)	4 strips (4x 4 tubes)	Qualitative standard tubes coated with 2 different amounts of *HSV*-1 and *HSV*-2 DNA, respectively, IC DNA and amplification enhancer	1
OT_AB	1	2	Optical tape	1
10x ROX	1x 0.50 ml	1x 0.50 ml	10x ROX passive reference dye	1



STORAGE CONDITIONS

The RoboGene® HSV DNA Qualitative Kit is delivered at room temperature except the Taq polymerase enzyme and PCR buffer which is shipped on dry ice. Store the HSV Qualitative Kit incl. Taq polymerase enzyme at -18°C – (-40°C) in the dark. The kit is stable until the expiration date when stored under these conditions.

IMPORTANT

An appropriate amount of Reagent mix (HSV_D4) should be dissolved in PCR grade water shortly before use. Remaining dissolved reagent mix can be stored at 2 - 8°C up to 14 days (do not freeze!). Always protect from light!

6 Necessary laboratory equipment and additives

- *HSV* positive samples (e.g. *HSV* standard strains Kupka and US cultured in Vero cells, obtained e.g. from the University Hospital Jena, Institute of Virology & Antiviral Treatment, German Reference Lab for HSV infections; provided Qualitative standards [HSV_D3_xx] may be considered as positive control
- *HSV* negative control (e.g. human plasma or serum free of *HSV* DNA)
- Rotor-Gene® 3000/6000, ABI PRISM® 7000/7300 SDS, MiniOpticon, CFX96, ABI® 7500 Fast, TOptical, qTOWER2&3 or LightCycler® 480 II
- Real-time instrument specific software for data analysis and reporting
- Suitable pipetting tools
- Micro centrifuge applicable for 0.2 ml and 1.5 ml tubes
- Vortex mixer
- 1.5 ml tubes
- Sterile pipette aerosol-barrier tips
- Applicator for optical tape and compression pad (for application to ABI PRISM® 7000/7300 SDS)
- Precision plate holder for tube strips (for application to 7500 Fast Real Time PCR System)
- Adapter Plate for tube strips (for application to LightCycler® 480)

7 Procedure

7.1 Collection and handling of clinical samples

- Serum, EDTA blood, liquor, blister aspirates, tissue biopsies, and swabs of lesions, rashes or ulcers should be collected in sterile tubes or syringes
- Analyze samples within one day
- Samples may be transported at room temperature, do not exceed the time of 6 hours after sample collection
- Sample *HSV* DNA may be stored deeply frozen for several months at -20 °C to -70 °C, stability depending on the storage temperature

7.2 HSV DNA purification from clinical samples

For nucleic acid purification and further analysis with the RoboGene® HSV DNA Qualitative Kit the INSTANT Virus RNA/DNA Kit (Analytik Jena, Order number: 847-0259200602) is recommended. Perform the *HSV* DNA purification steps according to the instructions of the INSTANT Virus RNA/DNA Kit manual.

NOTE
Include at least one replicate each of positive control plasma, negative control plasma, and NTC per run. Control plasma is not contained in the kit.

7.3 Internal DNA Control

The RoboGene® HSV DNA Qualitative Kit is provided together with stabilized internal control DNA (IC DNA). The IC DNA is contained in the extraction tubes which are stably coated with IC DNA and carrier nucleic ac-

id, respectively. The tubes are labelled with DNA_D1 and are contained in box 2 of the kit.

Applying the IC DNA containing extraction tubes together with the DNA extraction kit of choice always allows controlling for extraction yield, inhibitor load and judging the efficiencies of DNA extraction and subsequent PCR amplification, respectively. False-negatives due to failed extraction or excess of inhibitors in the sample may be excluded when getting positive amplification results for the internal control.

In case of using the INSTANT Virus RNA/DNA Kit or any other virus DNA extraction kit replace the original lysis tubes labelled with “Extraction tubes” by the extraction tubes (DNA_D1) contained in box 2. To consider the purification successful, the Ct value of the IC DNA purified together with *HSV* negative plasma or serum should be in the instrument specific ranges summarized in Table 15.

NOTE
Please add at first the lysis solution contained in the respective DNA purification kit to the extraction tubes containing the IC DNA and subsequently the patient sample. Do not add the sample directly to the extraction tube. Since the IC DNA is already contained in the extraction tube no additional pipetting steps are required.

7.4 General procedure of qualitative analysis

The qualitative standards are provided as ready-to use standard strips which are stably coated with defined amounts of *HSV-1* and *HSV-2* standard DNA.

NOTE
Please notice that the standard values are dependent on the DNA purification kit used together with the RoboGene® HSV DNA Qualitative Kit. We guarantee correct results only when using one of the recommended DNA purification kits.

NOTE

Please notice also that individual values may be below the stated detection limit of the kit. Detection limit of e.g. 50 IU/ml means that from the statistical point of view at least 95% of samples containing 50 IU per ml are correctly detected with the kit at a probability of error of 5%. This means on the other hand that values below the stated detection limit may be plausible but with an unacceptable high probability of error!

8 Protocol

8.1 Preparation of 5x Reagent Mix (HSV_D4, 5x)

1. Centrifuge the **HSV_D4** briefly at full speed to collect the lyophilized Reagent Mix on the bottom of the tube.
2. Add 175 µl **PCR grade water DNA** to **HSV_D4**; close the tube, mix by brief vortexing followed by brief centrifugation at full speed.
3. Incubate at 37 °C for 20 min using a thermal mixer (800 - 1,000 rpm), mix by brief vortexing followed by brief centrifugation at full speed.

NOTE
Dissolved reagent mix can be stored at 2-8 °C and always protected from light up to 14 days (do not freeze!)

8.2 Preparation of 1x Master Mix

1. Prepare the 1x Master Mix according to the following table. Mix by vortexing for at least 3 sec followed by brief centrifugation.

Table 8: Composition of 1x Master Mix per reaction

Reagent	Volume for 1x reaction (µl)			Final concentration
	RotorGene® 3000/6000/Q, Mini Opticon, CFX96, TOpti-cal, qTOWER 2&3 and LightCycler® 480 II	ABI PRISM® 7000/7300 SDS	ABI® 7500 Fast	
PCR grade water	12.1	9.6	11.85	-
10x PCR buffer FS1	2.5	2.5	2.5	1x
HSV_D4, 5x	5.0	5.0	5.0	1x
10x ROX	-	2.5	0.25	1x
Taq Polymerase FS1 (5 U/µl)	0.4	0.4	0.4	2 U/reaction
Total	20	20	20	

2. Identify sample tubes (HSV_D2_xx) and standards (HSV_D3_xx) carefully and place them onto a suitable rack.

NOTE
Attention should be paid to correct orientation of standards.

3. Add 20 µl 1x Master Mix to sample tubes and each tube with standards.
4. Add 5 µl **PCR grade water DNA** to tubes that serve as NTC and to all quantitation standards containing the 1x Master Mix. Do not exceed a final reaction volume of 25 µl.

5. Add 5 µl of eluate from DNA isolation (e. g. using the INSTANT Virus RNA/DNA Kit) to the respective sample tubes containing the 1x Master Mix. Do not exceed a final reaction volume of 25 µl.

NOTE
In order to continue preparation of real-time PCR setup, please choose the corresponding real-time cycler and follow the instructions.

Rotor-Gene® 3000/6000/Q

6. Cover the tubes with attached caps.
7. Program the applied real-time PCR platforms as indicated in Table 9/Table 10 below and start the program.

Table 9: PCR program for Rotor-Gene® 3000

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	15 sec
	Annealing/Elongation*	59 °C	1 min
* Data acquisition: Fluorescence Detection (FAM; JOE)			

Table 10: PCR program for Rotor-Gene® 6000/Q

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	30 sec
	Annealing/Elongation*	59 °C	1:30 min
* Data acquisition: Fluorescence Detection (FAM; JOE)			

ABI PRISM® 7000/7300 SDS

6. Cut optical tape (OT_AB) according to the required size and cover sample and quantitation standard strips carefully. Prevent cut-

injuries! Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended.

7. Centrifuge rack with loaded strips at 200x g for 1 min.
8. Program the applied real-time PCR platforms as indicated in Table 11 below and start the program.

Table 11: PCR program for ABI PRISM® 7000/7300 SDS

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	30 sec
	Annealing/Elongation*	59 °C	1:30 min
* Data acquisition: Fluorescence Detection (FAM; VIC)			

TOptical, qTOWER 2&3 and LightCycler® 480 II

6. Cover the tubes with optical tape (OT_AB) according to the required size and cover sample and quantitation standard strips carefully. Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended.
7. Centrifuge reaction plate with tubes at 200x g for 1 min.
8. Program the applied real-time PCR platforms as indicated in below Table 12 and start the program.

Table 12: PCR program for TOptical, qTOWER 2&3 and LightCycler® 480 II

Cycle	Profile	Temperature	Time	Ramping
1	Taq activation	95 °C	4 min	3.5 °C/sec
45	Denaturation	95 °C	15 sec	3.5 °C/sec
	Annealing/Elongation*	59 °C	1:00 min	2.5 °C/sec
* Data acquisition: Fluorescence Detection (FAM; VIC)				

MiniOpticon, CFX 96 and ABI® 7500 Fast

6. Cover the tubes or cut optical tape (OT_AB) according to the required size. Cover sample and standard strips carefully. Prevent cut-injuries! Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended.

7. Centrifuge reaction plate with tubes at 200x g for 1 min.

8. Program the applied real-time PCR platforms as indicated in below Table 13 and start the program.

Table 13: PCR program for MiniOpticon, CFX96 and ABI® 7500 Fast

Cycle	Profile	Temperature	Time
1	Taq activation	95 °C	4 min
45	Denaturation	95 °C	15 sec
	Annealing/Elongation	59 °C	1:00 min
* Data acquisition: Fluorescence Detection (FAM; VIC)			

9 Data analysis

Each DNA amplification is associated with generation of a fluorescence signal measurable in FAM channel (for *HSV* DNA) or in VIC/JOE channel (for IC) resulting in a sigmoid growth curve (log scale). The data analysis is performed according to manufacturer's instructions of the real-time PCR instrument using the respective software. Check the obtained data to ensure that the run is valid and to interpret results (Table 14). *HSV* DNA is determined based upon the Ct values for the sample *HSV* DNA. *HSV* DNA concentration is expressed as positive or negative according to the obtained Ct value.

NOTE

Setting of threshold may markedly influence Ct values

- Rotor-Gene: select Dynamic tube: Yes; Slope correction: Yes; Ignore first: 2; No template control threshold: 5%; Threshold modus: manual, *HSV*: >0.03, IC DNA: 0.01-0.04
- ABI PRISM: select auto baseline and set threshold manually to > 0.05

Set the threshold for all not listed real-time PCR instruments in the exponential phase of the amplification curve according to the instructions of the specific real-time PCR instrument IFU.

Due to manufacturing reasons the amplification of 1 standard of the standard strip may fail and should be omitted from calculation. In such case no right for warranty of the whole product may be deduced

Table 14: Interpretation of the results

FAM channel	JOE/VIC channel [1)]	Interpretation
Interpretation of detection results		
x	x	valid, detection of sample *HSV* DNA
x	-	valid, detection of sample *HSV* DNA[1)]
-	x	valid, *HSV* negative sample
-	-	invalid, no amplification/detection at all, repeat run
x (< 10 copies/run)	x	below lower limit of detection range of test - no detection
x (> 1x 10^9 copies/run)	x	above detection range of test - dilute original sample with the respective *HSV* negative human plasma/serum or phosphate buffered saline and test once again

[1)]IC DNA-specific signal may be missing in presence of a *HSV* DNA concentrations of >1x10^6 copies per run due to competition as tested with synthetic *HSV* DNA.

Expected Ct value for IC DNA (VIC/JOE channel) should be less than 35.

Table 15: *HSV* DNA (copies) given in standard and expected Ct values for Rotor-Gene

Standard	HSV standard DNA [copies/tube]	Expected Ct value for Rotor-Gene
		Mean[1)]
HSV-1 (A)	10,000	22.3
HSV-1	100	29.3
HSV-2	10,000	22.7
HSV-2	100	29.8

[1)] Data valid only when the recommended data analysis settings are selected.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
No signal at all	
■ Fluorescence measurement not activated	Read the user guide of the real-time PCR device.
■ False channels selected	Select FAM channel for *HSV* DNA and VIC/JOE channel for IC DNA.
■ Incorrect cycling program	Check instrument settings, repeat run.
■ Incorrect application of the kit	Read instruction for use.
■ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Low fluorescence signal recorded for both target and IC, target copy number underestimated	
■ Target DNA degraded	Use DNase free consumables and reagents; store DNA immediately on ice after purification. Read instruction for use of the extraction kit.
■ Optical lenses contaminated (Rotor-Gene)	See chapter “Maintenance” of respective instrument brochure, alternatively clean lens once per month using absolute isopropanol and cotton swabs.
No or weak signal for IC DNA in HSV-negative sample	
■ Incorrect cycling program	Check instrument settings, repeat run.
■ Excess of inhibitors in the sample/ loss of DNA during extraction	Use the recommended extraction kit and follow exactly manufacturer’s instructions.
■ Incorrect sample material (e.g. heparinized or lipaemic plasma)	Request for fresh EDTA blood or serum.

■ Wrong sequence of reagent addition to extraction tube	Add lysis solution to extraction tubes prior to addition of the sample
■ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Unexpectedly low Ct values for IC DNA particularly with high standards or high viral load samples	
■ Cross talk between target and IC recording channels (especially VIC/JOE)	Calibrate instrument using pure fluorescence dyes
Non-sigmoidal growth curves of qualitative standards, unacceptable high deviation of Ct	
■ Frequent freezing/thawing or incorrect storage of dissolved reagent mix	Read IFU, check storage conditions, prepare new reagent mix.
■ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Different amplification behavior of sample HSV DNA and standards, non-parallel growth curves in exponential phase of reaction	
■ Excess of inhibitors in the sample	Use the recommended extraction kit, follow exactly the manufacturer's instructions; consult attending doctor for patient medication.
■ Incorrect sample material	Use recommended sample type.
FAM signal for HSV-negative samples / NTC recorded	
■ Contamination with *HSV* DNA or DNA amplicons	Repeat extraction and/or PCR with new reagents; decontaminate instruments and work space.

If you have any further questions which are not answered, please contact our technical service.

11 References

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Subject to changes in design and scope of delivery as well as further technical development!