LightMix® Kit for the detection of HSV-1/2 (EC)

Cat.-No. 40-0378-32 Change to universal Extraction control Target (^{n}ECT)

Kit with reagents for the simultaneous detection of *Herpes simplex* types 1 and 2 (*HSV-1/2*) DNA using the Roche Diagnostics LightCycler® 1.x / 2.0 / 480 / 480 II Instruments or cobas z 480 Analyzer.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

Instructions for use with the LightCycler® 1.x / 2.0 Instruments see pages 4-6
Instructions for use with the LightCycler® 480 Instruments / cobas z 480 Analyzer see pages pages 8-10

1. Introduction

Herpesviridae is a family of enveloped, linear, double-stranded DNA viruses. Once infected the virus will be not eradicated and remains, and may be reactivated any time.

Herpes simplex virus (HSV) primarily infects mucosal surfaces. The virus is neuroinvasive and establish latency in the nervous system. Type 1 (HSV-1 or HHV-1) causes herpes outbrakes known as cold sores or fever blisters and settles in the trigeminal ganglion. Type 2 (HSV-2 or HHV-2) is more often associated with genital herpes and sets up residence in the sacral ganglion at the base of the spine. About 70% of the population is infected with HSV-1 and about 20% with HSV-2. At least 65% of HSV-1 infected people have no symptoms, or symptoms are too mild to notice. Both types may recur, typically by stress, for HSV-1 commonly after sun (ultraviolet) light exposition, and spread even when no symptoms are present.

Most published Real-Time-PCR systems for the detection of HSV-1/2 target the glycoprotein B or D[1] genes, the thymidine kinase, or the polymerase gene. Conserved variations in the polymerase gene allow an identification of HSV-1 and 2 by running a melting curve analysis [2,3]. Up to 10% of all HSV isolates show slight variations in the polymerase gene, resulting in deviating 'atypical' melting temperatures [4]. In contrast to the published assays [2] this kit contains hybridization probes specific for HSV-1, with the advantage to report for both virus types nearly identical Cp values versus amount.

2. Description

This kit provides a fast and accurate system to detect and identify the target in a nucleic acid extract. A fragment from the viral pol gene of 214 bp for HSV-1 or 215 bp for HSV-2 is amplified with specific primers and detected with LightCycler® Red 640 (LC640) labeled hybridization probes (channel 640). Identification of the virus is achieved by a melting curve analysis and type-specific melting points (Tm).

The Control Reaction is based on an additional 278 bp long fragment amplified from Lambda DNA, detected with short LC640 labeled hybridization probes not visible during amplification, but showing a peak of about 47°C in the melting curve analysis. T his PCR has no visible impact on the HSV specific reaction and will even fail in the presence of higher amounts of viral target (1,000 copies and more).

The former internal control (IC) has been changed to a spiked extraction control (sEC) in order to monitor a successful extraction and demonstrate the ability to run the PCR (absence of inhibition).
We recommend to use the 'Extraction Control' procedure; in case that the former procedure shall be maintained the usage as IC is described. Target and control primer/probe sequences remained unchanged. The novel extraction control target ^{n}ECT (no. 30-0259) contains Lamdba and PhHV DNA and may be used also for other LightMix kits, without adding the ECT provided with the other kit(s).
Note: With a MagNA Pure Compact extraction the recovery rate for the EC target can be very low or the target DNA can even get lost.

The supplied standard row allows to determine the linear range of the reaction and to estimate the quantity of the target sequence in unknown samples.

The kit is tested with 'LightCycler® FastStart DNA Master HybProbe' only.

3. Set Contents

3 Vials with **blue** cap with primers and probes for 32 PCR reactions *HSV-1/2* and Control

1 Vial with colorless cap containing control DNA HSV-1 with 2.5 x 10^4 target equivalents per rxn
1 Vial with colorless cap containing control DNA HSV-2 with 2.5 x 10^4 target equivalents per rxn

1 Standard row with 6 lyophilized standards *HSV-1* from 10^1 to 10^6 target equivalents per rxn
1 Sealing foil for the standard row

1 Vial with **white** cap containing Extraction Control Target (^{n}ECT) with 4.8 x 10^6 copies (total)
1 Vial with **black** cap for preparation of the Negative Control (NTC)

1 Certificate of Analysis (CoA) with lot-specific data

4. Additional Reagents and Items Required

	Roche Diagnostics
LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments)	Cat.-No. 04 929 292 001
LightCycler® 480 Multiwell Plate 384, white (480 Instrument)	Cat.-No. 04 729 749 001
or LightCycler® 480 Multiwell Plate 96, white (480 Instrument)	Cat.-No. 04 729 692 001

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of 640 and F3 instead of channel 705. We recommend to upgrade to software version 4.10 or higher.

4.1. Optional Additional Reagents

High Pure Viral Nucleic Acid Kit	Cat.-No. 11 858 874 001
or High Pure PCR Template Preparation Kit	Cat.-No. 11 796 828 001
Extraction Target nECT	TIB Cat.-No. 30-0259-96

5. Product Characteristics

PCR results (activation, 45 cycles and melting curve) are obtained within 45 minutes with the capillary based LightCycler® 1.x / 2.0 Instruments and within 80 minutes with '480' plate based Instruments.

Sensitivity
These reagents detect 10 copies of *HSV-1/2* DNA using Roche 'LightCycler® FastStart DNA Master HybProbe' (in an exemplary system, using cloned targets as reference).

Measuring range
The linear measuring range of the assay is 10^2 to 10^6 copies of *HSV-1/2* DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe'.

Storage and Stability
- Lyophilized reagents are stable for at least 6 months after shipment when stored protected from light at room temperature (18-25°C). See expiry date o n the product label.
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days when stored protected from light and refrigerated (4°C).

6. Experimental Protocol

Start programming before preparing the solutions. See the instrument operator's manual for details.

6.1. Preparation of Parameter-Specific Reagents (PSR) and Control Reaction :

One reagent vial with a **blue** cap contains primers and probes to run 32 reactions of HSV-1/2 and the Control Reaction .

Check for the colored pellet, then **add 66 µl** PCR-grade water, mix (vortex) and spin down.

►**Use 2 µl** reagent for a 20 µl PCR reaction.

This solution is stable at least ten days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

6.2. Preparation of Extraction Control Target (ECT):

Add **1,200 µl** PCR-grade water to the vial with **white** cap containing the Extraction Control Target

6.3. Sample Preparation:

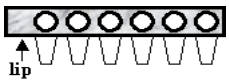
Before extraction add **10 µl** of ECT (vial **white** cap) to the sample. Skip if IC procedure is used.
Perform nucleic acid preparation as described in the protocol of the extraction kit used (**see 4.1**).

6.4. Preparation of No Template Extraction (NTC):

Add **900 µl** PCR-grade water to the **NTC** (vial **black** cap) and
add **100 µl** of ECT (vial **white** cap).

►Use **5 µl** No Template Control DNA for a 20 µl PCR reaction.

6.5. Preparation of the Standard Row and of the Control DNA :



The target DNA is provided in 6 different quantities to yield from 10^1 to 10^6 target molecules in 5 µl once resolved. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. Add **40 µl** of **NTC** (vial **black** cap) to each well. Use water if the IC procedure is selected. Mix the target DNA by pipetting the solution up and down 10 times.

Control DNA. Add 160 µl PCR-grade water to each vial with the colorless cap. Mix by pipetting the solution up and down 10 times (8 x 10^5 total copies, final concentration is 2.5 x 10^4 in 5 µl).

►**Use 5 µl** standard DNA for a 20 µl PCR reaction.

This standard solution is not long-term stable and will lose sensitivity under prolonged storage. After adding the target DNA to reaction mix, use the provided sealing foil to close the vials in order to avoid contaminations.

6.6. Preparation of the Reaction Mix

Include Positive Controls and at least one 'No Template Control' (NTC). In a cooled tube, prepare the reaction mix by multiplying the single reaction volumes by the number of reactions plus one reserve :

sEC Procedure	For use with the Roche FastStart Master	IC Procedure
Single reaction	**Component**	**Single reaction**
8.6 µl	Water, PCR-grade (colorless cap, provided with the Roche Master kit)	8.35 µl
2.4 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)	2.4 µl
2.0 µl	**Reagent** mix (parameter specific reagents, primers and probes, see **6.1.**)	2.0 µl
---- µl	ECT Control Target (vials **white** cap)	0.25 µl
2.0 µl	Roche Master (red cap, for preparation see Roche manual)	2.0 µl
15.0 µl	**Volume of reaction mix**	**15.0 µl**

Table 1

To run the assay without the Control Reaction substitute ECT with 0.25 µl PCR-grade water.

Mix gently, spin down and **transfer 15 µl** of the reaction mix to a capillary or well.

Add 5 µl of sample or standard to each capillary or well for a final reaction volume of 20 µl.
Close the capillaries / attach a foil to the multiwell plate and seal, and spin down.

Start run.

7. LightCycler® 1.x / 2.0 Instruments

7.1. Programming

The protocol consists of four program steps:

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [°C]	95	95	62	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:08	00:00:12	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Sec Target [°C]	-	-	55	-	-	-	-	-
Step Size [°C]	-	-	0.5	-	-	-	-	-
Step Delay (Cycles)	-	-	1	-	-	-	-	-
Acquisition Mode	None	None	Single	None	None	None	Cont	None

Table 2

7.2. Data Analysis

We recommend using the Second Derivative Maximum method (Automated (F" max)). The cycle number of the Crossing Point (Cp) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *HSV-1/2* data in channel 640 Quantification mode (LightCycler® 2.0 Instrument). The negative control (NTC) must show no signal. For the identification of the PCR product view *HSV-1/2* data in channel 640, Melting Curves.

For the Control Reaction view data in channel 640, melting analysis. The Control Reaction will show an amplification curve with a Tm at approximately 47°C.

The provided standard row is *HSV-1* target sequence DNA with concentrations (once dissolved) in the range from 10^6 to 10 copies per reaction should yield Cp values between 18 and 36 cycles (Cp values calculated with Second Derivative Maximum method).

For use in LightCycler® 1.x Instruments use channel F2 instead of channel 640 and channel F3 instead of channel 705 for detection.

7.3. Sample Data – Typical Results

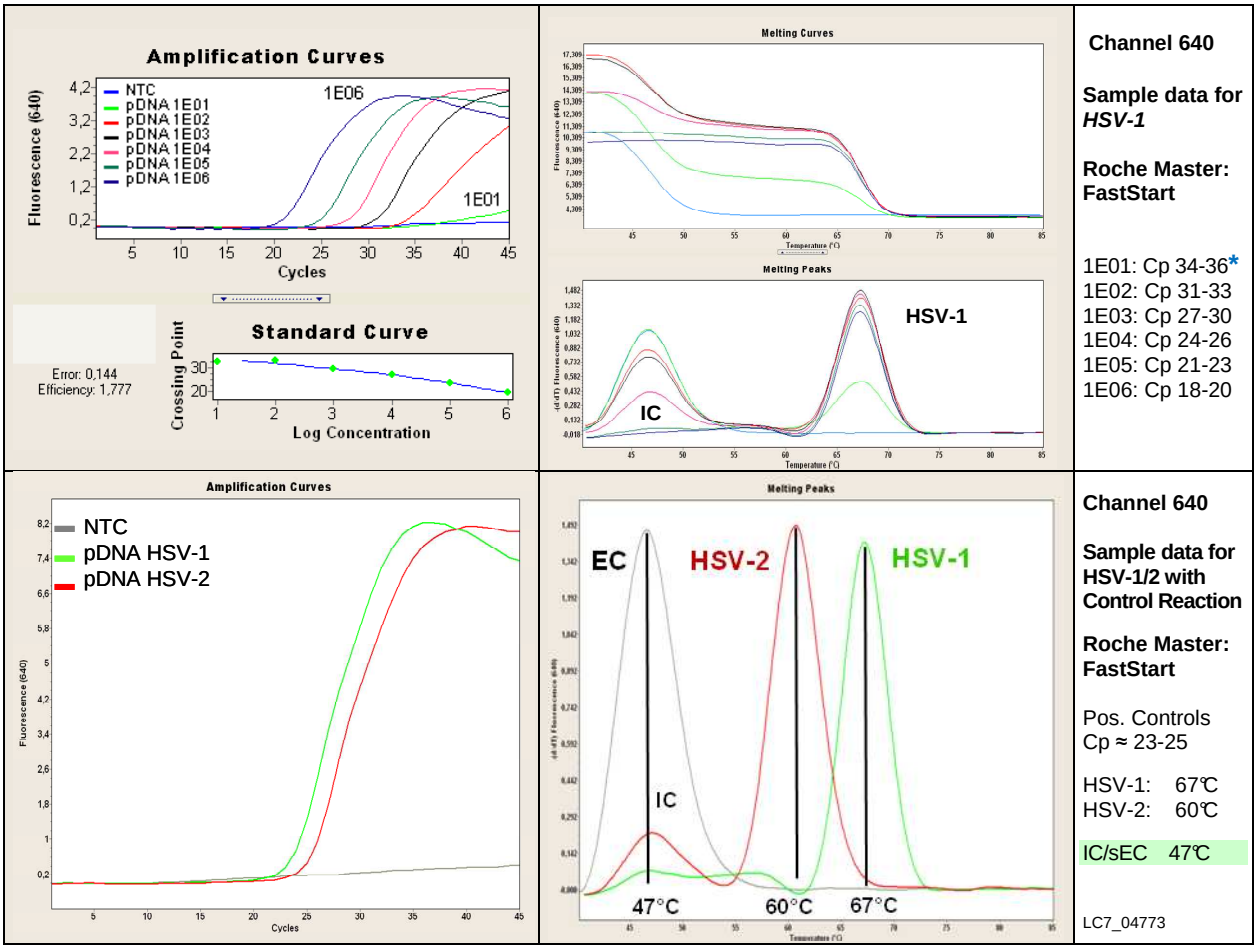


Fig.1. LightCycler® 2.0 sample data for the *HSV-1/2* detection system.

Upper panels: Left panel channel 640 quantification mode (Second Derivative Maximum) with amplification curves for HSV-1. Right panel channel 640 melting analysis for HSV-1 and the IC/sEC.
Lower panels: Left panel channel 640 quantification mode (Second Derivative Maximum) for HSV-1, HSV-2 and the IC/sEC. Right panel channel 640 melting analysis for HSV-1, HSV-2 and control reaction.

* **Notes:** Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The Cp values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta Cp). The Cp values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

The values of the respective melting temperatures (Tm) may vary ±2.5℃ between experiments. Samples wi th deviating curves should be subject to further investigations; sequence analysis can be provided by TIB Molbiol (contact service@tib-molbiol.de).

7.4. Interpretation of Data

Sample HSV-1/2	Control peak Tm ≈ 47℃	Peak 67°C (> 61℃)	Peak 60℃ (51-60℃)	NTC	Results (warnings)
no amplification	**detectable**	not detectable	not detectable	negative	**Negative (not detectable)**
Cp < 38 +	not relevant	**peak**	not detectable	negative	**Positive for HSV-1**
Cp < 38 +	not relevant	not detectable	**peak**	negative	**Positive for HSV-2**
Cp < 38 +	not relevant	**peak**	**peak**	negative	**Positive for HSV-1 and HSV-2**
no amplification	not detectable	not detectable	not detectable	negative	**PCR failure, repeat experiment**
not relevant	not relevant	not relevant	not relevant	positive	**Contamination, clean / repeat**

Table 3. Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: FastStart DNA HybProbe)

+ The cut off value is a recommendation only - this value has to be defined by the user. Compare with the lot specific values for 10 copies as reported in the Certificate of Analysis (CoA). 1-2 cycles later than for 10 copies corresponds to ~5 copies/reaction.

7.5. Intermediate Melting Peaks

About 5% off all HSV-positive isolates may yield peaks with divergent melting temperature, which can be explained with sequence variations in the probe binding region.

Melting points between the HSV-1 standard (67℃) an d HSV-2 (60℃) are considered as HSV-1 variants, whereas peaks at temperatures lower than the HSV-2 melting peak are most probable HSV-2 variants.

Figure 2 shows examples for atypical melting points :

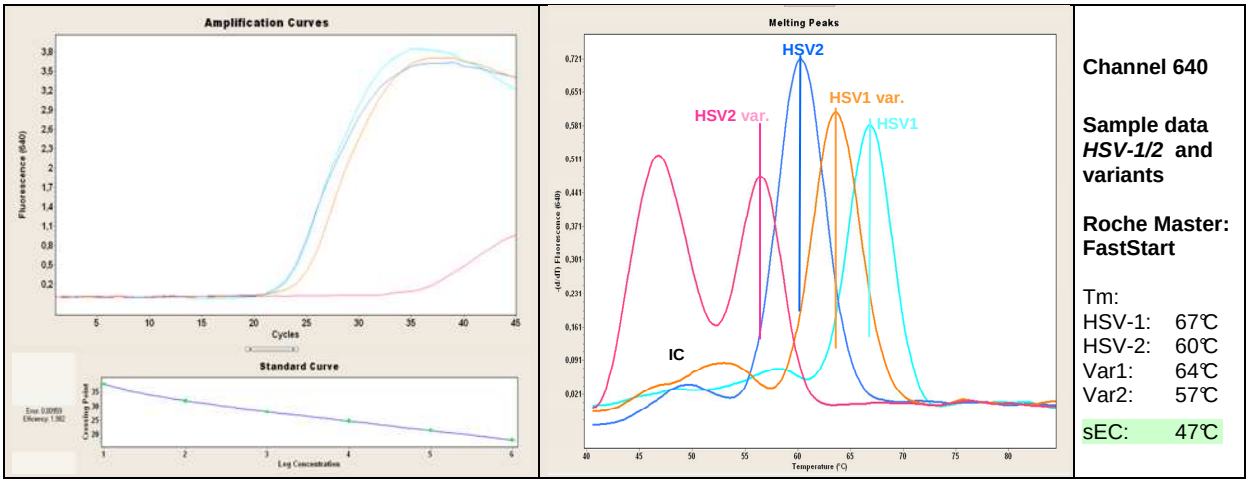


Fig.2. LightCycler® 2.0 sample data for *HSV-1/2* atypical melting.

Left panel. Channel 640 quantification mode (Second Derivative Maximum) with amplification curves for HSV-1/2 and variants. **Right panel.** Channel 640 melting analysis for HSV-1/2 controls and variants of HSV-1 and HSV-2.

Variant 1. The 64℃ peak (orange) has been observed with an isolate from Germany which is identical to Genbank *Acc.* No. X14112*, HHV- 1*.

Variant 2. The 57℃ peak (pink) has been observed with an is olate from Entebbe, Uganda (kindly provided by Dr. Peter Hughes). Within the amplified region this virus was 92% identical with HSV-2 and 90% identical to HSV-1, thus matching with neither type.

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8. LightCycler® 480 Instruments and cobas z 480 Analyzer

8.1. Programming

The protocol consists of four program steps:

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Detection Format:
LightCycler® 480 Instrument: 483-533, 483-640
LightCycler® 480 II Instrument: 465-510, 498-640
cobas z 480 Analyzer: 465-510, 498-645

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target [℃]	95	95	62	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:08	00:00:12	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [℃/s] **96**	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate [℃/s] **384**	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
Sec Target [℃]	-	-	55	-	-	-	-	-
Step Size [℃]	-	-	0.5	-	-	-	-	-
Step Delay (Cycles)	-	-	1	-	-	-	-	-
Acquisition Mode	None	None	Single	None	None	None	Continuous	None
Acquisitions [per ℃]	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**

Table 4

8.2. Data Analysis

Note: cobas z 480 Analyzer signal levels are about 50% compared to LightCycler® 480 II results.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (Cp) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *HSV-1/2* data with Filter Combination 498-640 Quantification mode. The negative control (NTC) must show no signal. For the identification of the PCR product view *HSV-1/2* data with Filter Combination 498-640, Melting Curves mode.

For the Control Reaction view Filter Combination 498-640, melting analysis. The Control Reaction will show an amplification curve with a Tm at approximately 48℃.

The provided standard row is *HSV-1* target sequence DNA with concentrations (once dissolved) in the range from 10^6 to 10 copies per reaction should yield Cp values between 18 and 36 cycles (Cp values calculated with Second Derivative Maximum method).

8.3. Sample Data – Typical Results

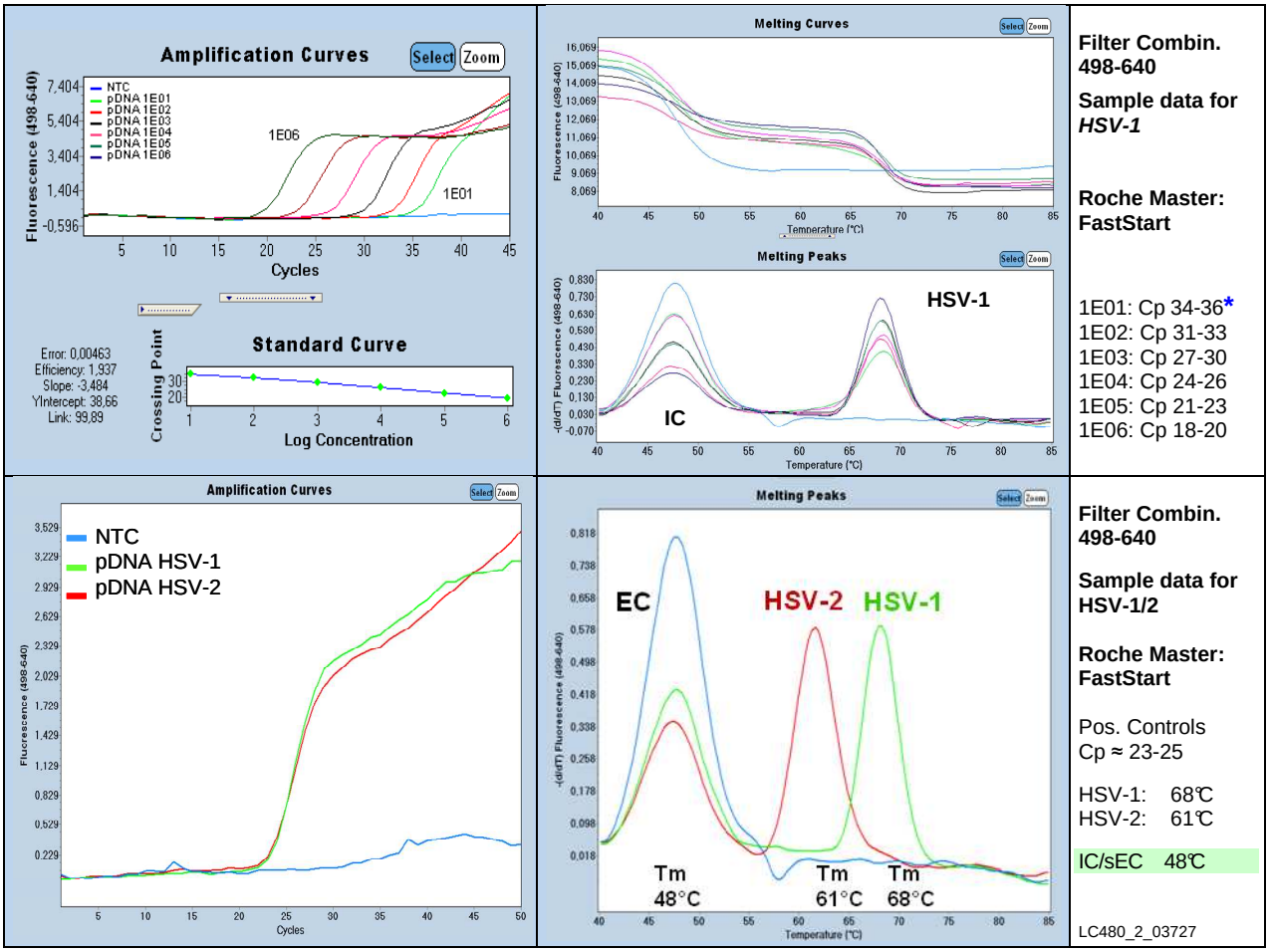

Fig.3. LightCycler® 480 II sample data for the *HSV-1/2* detection system.

Upper panels: Left panel Filter Combination 498-640 quantification mode (Second Derivative Maximum) with amplification curves for *HSV-1*. Right panel Filter Combination 498-640 melting analysis for HSV-1 and the IC/sEC.
Lower panels: Left panel Filter Combination 498-640 quantification mode (Second Derivative Maximum) with amplification curves for Control HSV-1, Control HSV-2 and the IC/sEC.
Right panel: Melting analysis for HSV-1, HSV-2 and the Control Reaction, Filter Combination 498-640

* **Notes:** Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The Cp values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta Cp). The Cp values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

The values of the respective melting temperatures (Tm) may vary ±2.5℃ between experiments. Samples wi th deviating curves should be subject to further investigations; sequence analysis can be provided by TIB Molbiol (contact service@tib-molbiol.de).

8.4. Interpretation of Data

Sample 640 HSV-1/2	Control peak Tm ≈ 48℃	Peak 68℃ (> 61℃)	Peak 61℃ (51-61℃)	NTC	Results (warnings)
no amplification	**detectable**	not detectable	not detectable	negative	**Negative (not detectable)**
Cp < 38 +	not relevant	**peak**	not detectable	negative	**Positive for HSV-1**
Cp < 38 +	not relevant	not detectable	**peak**	negative	**Positive for HSV-2**
Cp < 38 +	not relevant	**peak**	**peak**	negative	**Positive for HSV-1 and HSV-2**
no amplification	not detectable	not detectable	not detectable	negative	**PCR failure, repeat experiment**
not relevant	not relevant	not relevant	not relevant	positive	**Contamination, clean / repeat**

Table 5. Typical analysis results (Plate-based 480 instruments, Roche Master: FastStart DNA HybProbe)

+ The cut off value is a recommendation only - this value has to be defined by the user. Compare with the lot specific values for 10 copies as reported in the Certificate of Analysis (CoA). 1-2 cycles later than for 10 copies corresponds to ~5 copies/reaction.

8.5. Intermediate Melting Peaks

About 5% off all HSV-positive isolates may yield peaks with divergent melting temperature, which can be explained with sequence variations in the probe binding region.

Melting points between the HSV-1 standard (68℃) an d HSV-2 (61℃) are considered as HSV-1 variants, whereas peaks at temperatures lower than the HSV-2 melting peak are most probable HSV-2 variants.

Figure 2 shows examples for atypical melting points :

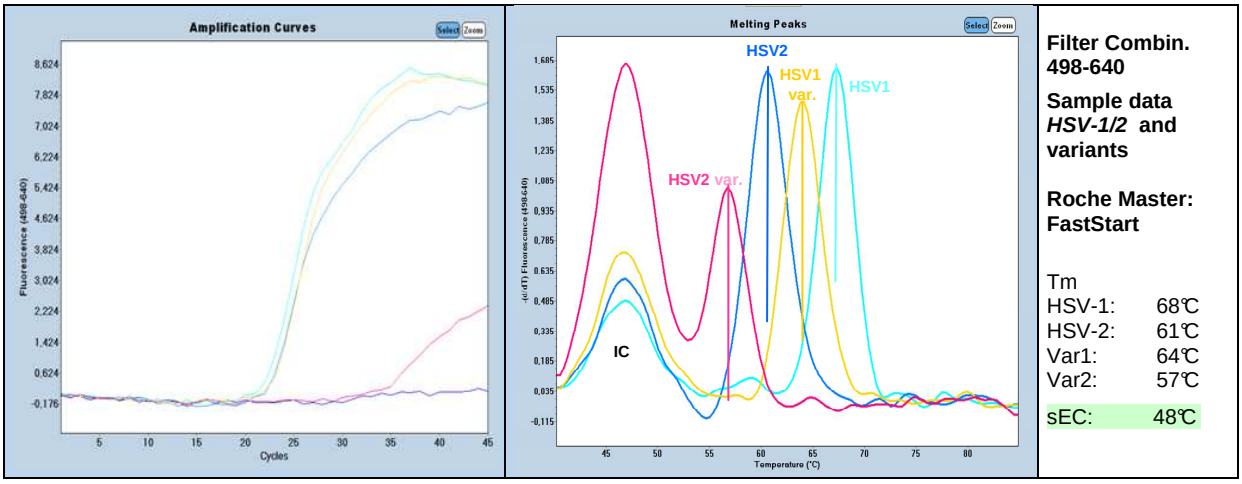

Fig.4. LightCycler® 480 II sample data for the *HSV-1/2* atypical melting.

Left panel. Filter Combination 498-640 quantification mode (Second Derivative Maximum) for HSV-1/2 controls and variants. **Right panel.** Filter Combination 498-640 melting peaks for HSV-1/2 controls and variants of HSV-1 and 2.

Variant 1. The 64℃ peak (yellow) has been observed with an isolate from Germany which is identical to Genbank *Acc.* No. X14112, *HHV- 1*.

Variant 2. The 57℃ peak (pink) has been observed with an is olate from Entebbe, Uganda (kindly provided by Dr. Peter Hughes). Within the amplified region this virus was 92% identical with HSV-2 and 90% identical to HSV-1, thus matching with neither type.

9. Conversion Factor

The amount of virus per sample (Viral Load) is usually reported as copies / ml while PCR reports copies per reaction. The conversion factor between both numbers depends on sample and extraction volumes. Extraction starts usually from less than one milliliter and PCR test does not use all extracted material.

The viral load (VL) can be calculated using the following general formula:

VL [copies/ml] = MV x EVF x SF

where:

VL = **Viral Load**
MV = **M**easured **V**alue [copy number per reaction]
EVF = **E**xtraction **V**olume **F**actor [Final extraction volume / PCR sample volume]
SF = **S**ample **F**actor [1,000 µl / extracted volume of clinical sample]

For example, extracting from 200µl of clinical sample results in a correction factor of 5. Using 5 µl extract from a total elution volume of 100 µl results in a correction factor of x 20, resulting in a conversion factor of 100.

VL [copies/ml] = Measured **V**alue [copy number per reaction] **x 100**

Note: For clinical samples different from body fluids the amount of sample is dependent from the collecting technique (swab, tissue, FFPE) and the viral load can not be related to the volume.

10. Control Reaction - Adjusting the Inhibition Control

The Control Reaction is based on a melting curve analysis only, and will not detect a gradual inhibition causing a delay of amplification only, which would otherwise be detected based on delayed Cp values.

These instructions are based on the use of about 1000 copies of the Extraction Control Target (ECT) per reaction. In the Negative Control reaction the Control will be visible after about 33 thermocycles.

In order to detect a delay of five cycles or more due to inhibitory substances introduced through the clinical samples the Control Target can be diluted to amplify later. This manual does not include detailed instructions nor the recommended dilution, because the kit can be used in conjunction with different extractions kits, volumes and procedures (see section 9), with have an influence on the amount of Control target present in the extract, the amplification, and the respective Cp values.

10.1 Procedure for Adjusting of the Control Reaction :

10.1.1 Dilute the ECT three times in 1:2 steps to use 840 to 105 copies per reaction, add to aliquots of a negative-tested clinical sample, and extract according to the normal procedure. If the IC procedure is used, dilute the NTC solution accordingly, and add the dilutions to the PCR mixture (section 6.7).

10.1.2 Run the PCR as described in section 7.1 or 8.1 with 33 instead of 45 cycles followed by a melting curve analysis, and add four times each three cycles followed by a melting curve analysis.

Record the results in the melting curve analysis after 33, 36, 39, 42 and 45 cycles (yes / no peak).

10.1.3 Use the amount of ECT which is visible after 39 cycles but not visible after 36 cycles to be able to detect a delay of six cycles (by calculation this is a 64-fold reduction of sensitivity).

Note: The Control Reaction assay used here is less effective compared to the analytical assay and thus more sensitive on eventual PCR inhibition.

11. References

[1] Preliminary Comparison of Three LightCycler PCR Assays for the Detection of Herpes Simplex Virus in Swab Specimens. Whiley DM, Syrmis MW, Mackay IM, Sloots TP. Eur J Clin Microbiol Infect Dis. 2003

[2] Espy MJ, Uhl JR, Mitchell PS, Thorvilson JN, Svien KA, Wold AD, Smith TF. Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. J Clin Microbiol.38 (2000) 795-799

[3] Detection and subtyping of Herpes simplex virus in clinical samples by LightCycler PCR, enzyme immunoassay and cell culture. Burrows, J., Nitsche, A. Bayly, B., Walker, E. Higgins, G., and Kok, TW. BMC Microbiology 2002, 2:12

[4] Failure To Genotype Herpes Simplex Virus by Real-Time PCR Assay and Melting Curve Analysis Due to Sequence Variation within Probe Binding Sites. Anderson TP, Werno AM, Beynon KA, Murdoch DR. JCM 41 (2003) 2135-2137

12. Material Safety Data

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008 (1999)] and the European Union Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

12. Version History

Red notes mark events require changed procedures, blue mod. sequences

V080226	Release version
V100823	16 rxn per vial
V110216	Change to 32 rxn per vial
V120118	Implementation of version history
V130314	Probe for detection of the Internal Control shortened in order to avoid traces of signals in the amplification in channel 640 Melting curve shows a lower Tm for the Internal Control. Chapter 4: Roche Color Compensation kit reference removed Chapter 8: Instrument cobas z 480 included. Chapter 9: Conversion Factor introduced.
V130813	Editorial changes
V140909	Change Internal Control (IC) to spiked Extraction Control (sEC) Section 10 Control Reaction inserted
V150707	Change to universal ^{n}ECT target containing Lambda and PhHV DNA

Roche SAP order n° 05943647001

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.