Instructions For Use

LightMix® Modular HSV (Herpes-Simplex Virus 1/2) FAM

Cat.-No. 53-0133-96 Roche SAP n° 00 000 000 000

Kit with reagents for 96 PCR reactions 20 µl for detection of HSV-1/2 [lyophilized]

1. Content Storage and Expiry

Storage at Arrival:

1 Vial yellow cap 96 reactions HSV-1/2 (lyophilized)
1 Vial black cap Positive Control(≈ Cp 30), lyophilized

Store cooled or at ambient temperature
Do not freeze the lyophilized reagents.

- Kits are stable for one year after production (store 4°C to 25°C in the dark). See lot-specific expiry date.
- Reconstituted reagents are stable for two weeks if stored protected from light and cooled (2°C to 8°C).
- Dissolved reagent can be stored long-term if frozen (-15°C to -25°C). Avoid multiple freeze-thaw cycles.
- Reconstituted positive controls must be stored frozen. Minimize multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex DNA Master Cat.-No. 07 339 585 001

3. 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 establishes latency in the nervous system. Type 1 (HSV-1 or HHV-1) causes herpes outbreaks 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.

4. Description

A 64 bp long fragment from the viral polymerase gene is amplified with specific primers and detected with a FAM labelled hydrolysis probe (530 channel).

5. Specification

This assay detects 10 genome equivalent copies or less per reaction.

6. Sample Material and Extraction

Typical sample type are vesicular lesions, cerebrospinal fluid, genital and oral swabs, whole blood or plasma. For extraction protocols see Roche MagNA Pure or Roche manual kit instructions.

7. Material Safety Data (MSDS)

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

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

8. Instructions for Use

When run in combination with assays with other fluorophores (channels), a Color Compensation file must be applied. To generate a Color Compensation file see instructions in the ***Roche 06296971001 Universal Color Compensation Hexaplex*** Instructions For Use.

8.1. Programming Roche 480 Instruments

See the Instrument operator's manual for details. Start programming before preparing the solutions. The protocol consists of three program steps:

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification
- 3: Cooling: cooling the instrument

Detection Format 530 Channel	**Set Quant Factor 10, Max Integration Time 1 sec**
LightCycler® 480 Instrument:	483-533
LightCycler® 480 II Instrument:	465-510
cobas z 480 Analyzer (open channel):	465-510

Program Step:	*RT Step**	***Denaturation***	***Cycling***			***Cooling***
Parameter						
Analysis Mode	*None*	None	Quantification mode			None
Cycles	*1*	1	45			1
Target [°C]	55	95	95	60	72	40
Hold [hh:mm:ss]	00:05:00	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	**Single**	None	None

* *optional use if combining with 1-Step RT-PCR*

Table 1

8.2. Experimental Protocol

- **Sample material:** Use aqueous nucleic acid preparations (e.g. 'High Pure PCR Template Preparation Kit').
- **Negative control:** Always run at least one no-template control (NTC) - replace the template NA with water.
- **Positive control:** Run a positive control - replace the template NA with the provided Positive Control.

For an increased sensitivity use 10 µl nucleic acid per 20 µl reaction, for sample types were inhibition may occur e.g. Fecal sample extracts, use 5 µl. For 10 µl reactions in 384 well plates use 5 µl /2.5 µl.

8.2.1. Preparation of Parameter-Specific Reagents (PSR, 96 reactions):

The reagent vial with a **yellow** cap contains all primers and probes to run 96+ LightCycler® reactions.

Check for the colored pellet, then **add 50 µl** PCR-grade water, mix (vortex) and spin down.
For robotic pipetting the volume can be extended to 55 µl (signals will decrease by 10-20%).

►**Use 0.5 µl** reagent for a 20 µl PCR reaction.

8.2.2. Preparation of the Positive Control DNA

Add 160 µl RNase/DNase-free 10 mM Tris buffer pH 8 - 8.5 to the vial with the **black** cap, if using 10 µl sample volume add **320 µl**. Mix by pipetting up and down 10 times. If vortexing spin down to collect the solution. Store dissolved controls frozen. Use of Tris increases the stability in solution.

Notes: Opening this vial may cause contamination of the workspace. Pulse spin vial prior to opening.

►**Use 5 µl** positive control (≈ Cp 30) for a 20 µl PCR reaction (10 µl if using 10 µl sample volume).

8.2.3. Preparation of the Reaction Mix

Multiply volumes by the number of reactions plus controls and one reserve, prepare in a cooled tube:

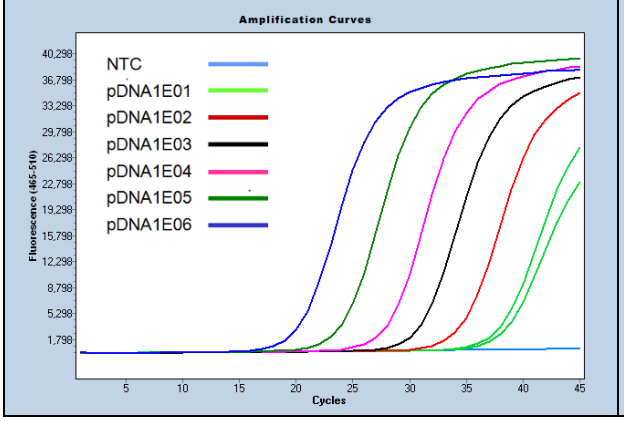
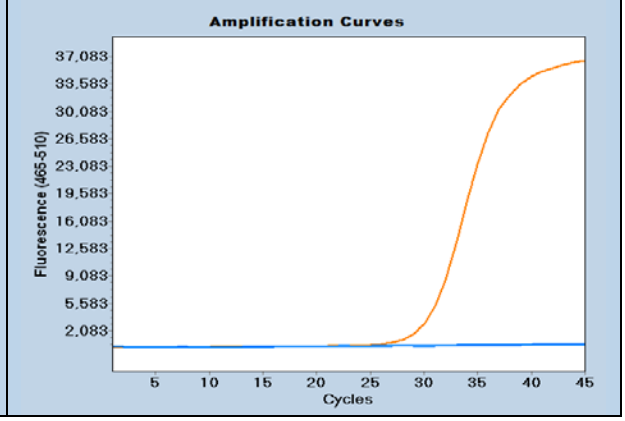
For use with the Roche LightCycler® Multiplex DNA Master		
for 5 µl extract	**Component**	**10 µl extract**
10.5 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.5 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 µl**

Table 2

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.

Add 5 µl (10 µl) of sample or control to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.
Start run

9. Typical Results (Data from LightCycler® 480 II system)

Channel 530 : HSV	Positive Control
	

Dilution row 10E6 to 10 copies / reaction

Figure 1

10. Reading the Results

Perform data analysis as described in the operator's manual. For multiplex assays select the color compensation. We recommend using the Second Derivative Maximum method (Automated (F" max). View results in the FAM channel. The negative control (NTC) must show no signal.

Channel 530 (sample)	Channel 660 Control Reaction	Channel 530 NTC Control	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 39+	**Not relevant**	**Negative**	**HSV Positive**
No amplification	**Not detectable**	**Not relevant**	**PCR failure Repeat**
Amplification signal	**Not relevant**	**Positive**	**Contamination Repeat**

Note: cobas z 480 Analyzer signal levels are ~ 50% as compared to LightCycler® 480 II results.
+ Recommendation: Define the cut-off 2-4 cycles higher than observed Cp value for 10 copies.

11. References

12. Multiplex PCR Compatibility

The HSV assay can be combined with other assays up 6plex PCR including an internal control (IC) or a spiked extraction control (e.g. PhHV or MSTN) in the 660 channel as depicted below:

Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
	HSV					X	X	X	X	X
	HSV	VZV			MSTN or PhHV	X	X	X		
	HSV	VZV	EBV			X	X			
CMV	HSV	VZV	EBV	HHV-6		X				

Table 3

Alternative products for HSV type identification are 53-0135-96HSV-1 and 64-0136-96 HSV-2.

13. Version History

V151001	Release Version	2015-10-01
V160313	1. Storage of controls, 8.2.2 buffer, 8.2.3 wording	2016-08-18
V190123	Editorial changes, Cp ranges updated, 8.2.2 Use Tris buffer	2019-01-31

Certificate of Analysis (CoA)

Lot n° **3571**

Expiry :

Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	18-20	22-24	25-27	28-30	31-33	34-37	
Measured							✓
Signal level				35-50			
Measured							✓
Negatives	**10/10**						✓

Note: Fluorescence (FL) levels depend on instrument settings and may vary. The crossing point (Cp) values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (ΔCp).

QC Acceptance Date: YYYY-MM-DD

We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.

Name(s) :

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