Instructions For Use

LightMix® Modular Herpes-simplex Virus-1 530

Cat.-No. 53-0135-96 Roche SAP 08 708 606 001-AU

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

1. Content Storage and Expiry

1 Vial yellow cap 96 reactions HSV-1 (lyophilized)
1 Vial black cap Positive Control (≈ Cp 30), lyophilized)
1 Standard row with 10 to 10^6 target equivalents per rxn

Storage at Arrival:

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. About 70% of the population is infected with HSV-1.

HSV-1 infected people have no symptoms, or symptoms are too mild to notice. HSV-1 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 77 bp long fragment from the glycoprotein G 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 to combine 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.
Add 50 µl PCR-grade water to each reagent vial, mix the solution (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

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 Standard Row

The target DNA is provided in 6 different quantities to yield from 10^1 to10^6 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** PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution ten times up and down.

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

8.2.4. Preparation of the Reaction Mix

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

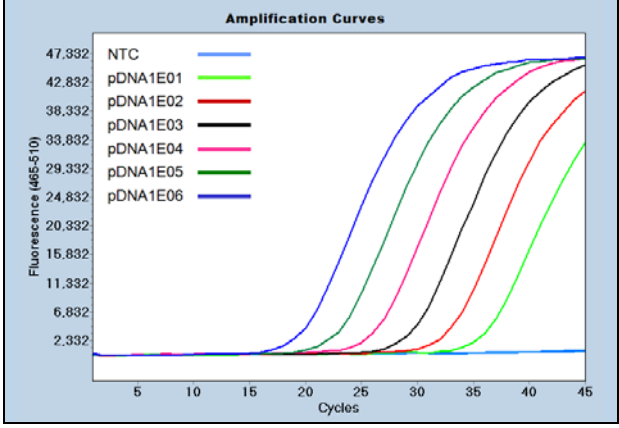
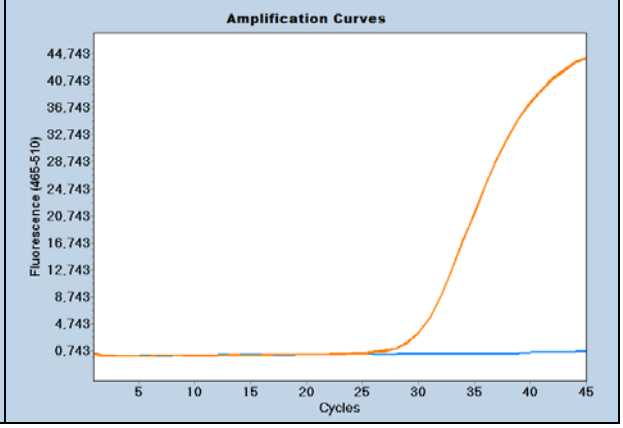
For use with the Roche LightCycler® Multiplex DNA Master		
for 5 µl extract	**Component**	**10 µl extract**
10.5 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.5 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction 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-1	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-1 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

(none)

12. Multiplex PCR Compatibility

The HSV-1 assay can be combined with HSV-2 assays 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
	HSV-1				PhHV or MSTN
	HSV-1	VZV		HSV-2	
CMV	HSV-1	VZV	EBV	HSV-2	

480 II	z 480	LC96	LC2.0	Nano
X	X			
X	X			
X				

Table 3

13. Version History

V170830	Release Version	2017-08-30
V180424	CoA acceptance criteria updated. 1. Clarification storage conditions	2018-04-24
V180909	Editorial changes	2018-09-09

Certificate of Analysis (CoA)

Lot n°
Expiry :

Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	19-21	22-24	26-28	29-31	32-34	36-38	
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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