

LightMix[®] Modular Orthopox Virus

530

Cat.-No. 53-0548-96

Roche SAP n° 09 796 347 001

Kit with reagents for 96 PCR reactions 20 µl for detection of OPXV [lyophilized]

1. Content, Storage and Expiry

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

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

Orthopoxviruses (OPXV) are very large (200 nm like small bacteria), brick-shaped ds DNA viruses with a genome of ~ 200 kb. They cause febrile illnesses with prominent vesicular rash.

Smallpox (variola) causes severe disease in man but has been eliminated by vaccination using the **Vaccinia** pox virus. **Cowpox** is acquired by humans from milking cows.

Monkey pox is a rare smallpox-like disease of children in central Africa. Typical signs are fever, sore throat, headache and vesiculopustular rash, but also bronchopneumonia and diarrhea. The case fatality rate is more than 10%. Monkey pox related to MSM communities has been recently reported in different countries in Europe and USA.

4. Description

A 113 bp long fragment from the 14 kDa gene from the virus is amplified with specific primers and detected with a FAM labeled hydrolysis probe.

5. Specification

This assay detects 10 genome equivalent copies or less per reaction (plasmid DNA dilution).

6. Sample Material and Extraction

Typical samples are swabs from skin lesions.

For extraction protocols see Roche MagNA Pure or Roche manual kit instructions.

7. Material Safety Data (MSDS)

This product is not hazardous (according to regulation (EC) No 1272/2008), not toxic, not IATA-restricted. Not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and EU Directives (EC) No 1907/2006 and (EC) No 2015/830 any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a MSDS.



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 (default setting is 1)
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 where 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 the primers and probe 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 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

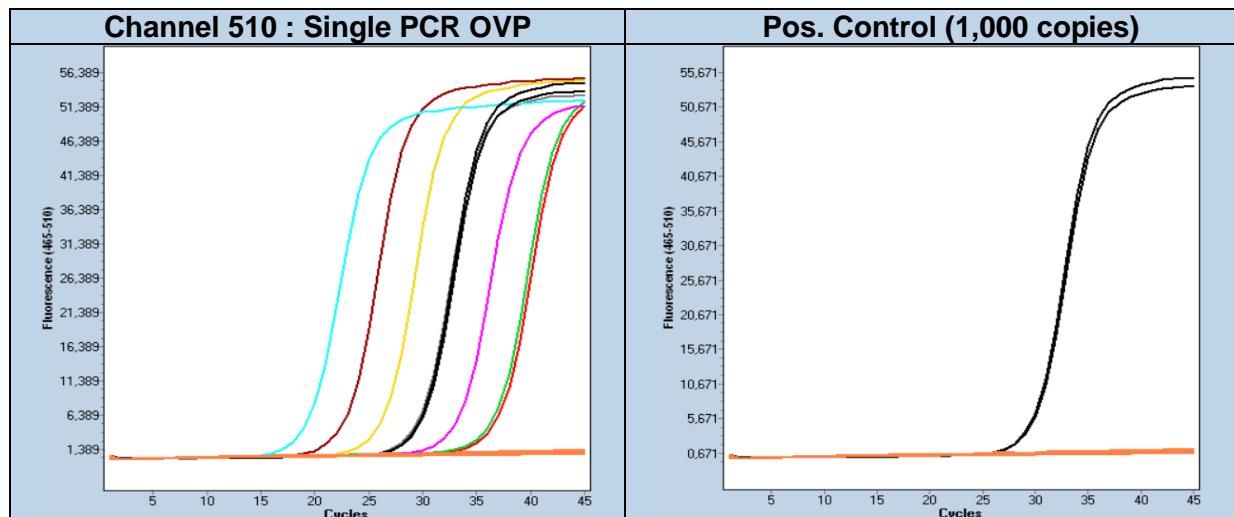
Table 2

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)



Dilution row 1E6 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 < 37 +	Not relevant	Negative	Orthopox virus 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 1-2 cycles higher than observed Cp value for 10 copies.

11. References

Real-time PCR system for detection of orthopoxviruses and simultaneous identification of smallpox virus. Olson VA, Laue T, Laker MT, Babkin IV, Drosten C, Shchelkunov SN, Niedrig M, Damon IK, Meyer H. J Clin Microbiol. 2004 May;42(5):1940-6

12. Multiplex PCR Compatibility

This assay can be combined with an internal control (IC) or a spiked extraction control (for example PhHV) as depicted below :

EHEC Multiplex PCR and Instrument Compatibility						480 II	z 480	LC96	LC2.0	Nano
Color Compensation 40-0320 is mandatory for Multiplex PCR										
500	530	580	610	640	660					
	Orthopox	control				X	X	X	X	X
	Orthopox				PhHV	X				


Table 3

13. Version History

V220519 Release version

2022-05-19

Note. EU / German Export Restrictions for this product (Dual Use Bioweapon Detection). End-user-certificate may be required. End user will be reported to the National Authorities

Certificate of Analysis (CoA)							
Lot n° 5229							
Expiry : YYYY-MM-DD							
Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	19-21	22-24	25-27	29-31	32-34	35-37	
Measured							✓
Signal level				40-60			
Measured							✓
Negatives	10/10						✓
<p>Note: Cp (crossing point) values collected with pDNA (single target PCR). Fluorescence (FL) levels depend on instrument settings and may vary. 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 (ΔCp).</p>							
DOM (manufactured): YYYY-MM-DD				QC Acceptance: 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) :							
<i>Name1</i>				<i>Name2</i>			

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