



For life science research use only. Not for use in diagnostic procedures. For *in vitro* use only.



MOLBIOL

580

# LightMix® Modular Monkeypox Virus

Cat.-No. 58-0550-96

Roche SAP n° 09 798 471 001

Kit with reagents for 96 PCR reactions 20 µl for detection of MPXV

## 1. Content, Storage and Expiry

- 1 Vial red cap 96 reactions MPXV (dried)
- 1 Vial black cap Positive Control (dried)

## 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 reagent is 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  
or 1-step RT polymerase

Cat.-No. 07 339 585 001  
90-9999-96

*We strongly recommend to use LightCycler® Multiplex DNA Master, only if this reagent is not available, 1-step RT polymerase can be used as substitute. For optimal performance with 1-step RT polymerase we recommend to adjust the annealing T<sub>m</sub> from 60 to 64°C, as described in section 8.1. Nevertheless, a differing performance compared to the amplification shown in section 9 is typical.*

## 3. Introduction

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

Orthopoxviruses that are known to cause disease in humans include smallpox, vaccinia virus, cowpox, and **monkeypox**.

**Monkeypox** 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 diarrhoea. Monkeypox related cases have been recently reported in different countries in Europe and USA.

## 4. Description

A 106 bp long fragment of the J2L/J2R gene (TNF receptor) and a 98 bp long fragment of the B7R gene from monkeypox virus are amplified with specific primers and detected with R6G labeled hydrolysis probes. With this dual-target approach a potential deletion of the TNF receptor gene is compensated.

Positive control is a synthetic cloned fragment (shorter than 500 bases / no genomic DNA contained).

## 5. Specification

This assay detects at least 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

<b>Detection Format 580 Channel</b>	<b>Set Quant Factor 10</b> (default setting is 1)
LightCycler® 480 Instrument:	523-568
LightCycler® 480 II Instrument:	533-580
cobas z 480 Analyzer (open channel):	540-580

Program Step:	RT Step*	Denaturation	Cycling			Cooling
<b>Parameter</b>						
Analysis Mode	None	None	Quantification mode			None
Cycles	1	1	45			1
Target [°C]	55	95	95	60 <sup>†</sup>	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] <b>96</b>	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] <b>384</b>	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 assays

Table 1

<sup>†</sup> with 1-step RT polymerase use 64°C

### 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 **red** 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 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:

LightCycler® Multiplex DNA Virus Master		1-step RT Polymerase 90-9999-96 *	
10.5 µl	Water, PCR-grade	Water, PCR-grade	4.5 µl
0.5 µl	Reagent mix (PSR)	Reagent mix (PSR)	0.5 µl
--	Control Reaction (Multiplex PCR)	Control Reaction (Multiplex PCR)	--
4.0 µl	Roche Master	1-step RT Master	10.0 µl
<b>15.0 µl</b>		<b>15.0 µl</b>	

Table 2

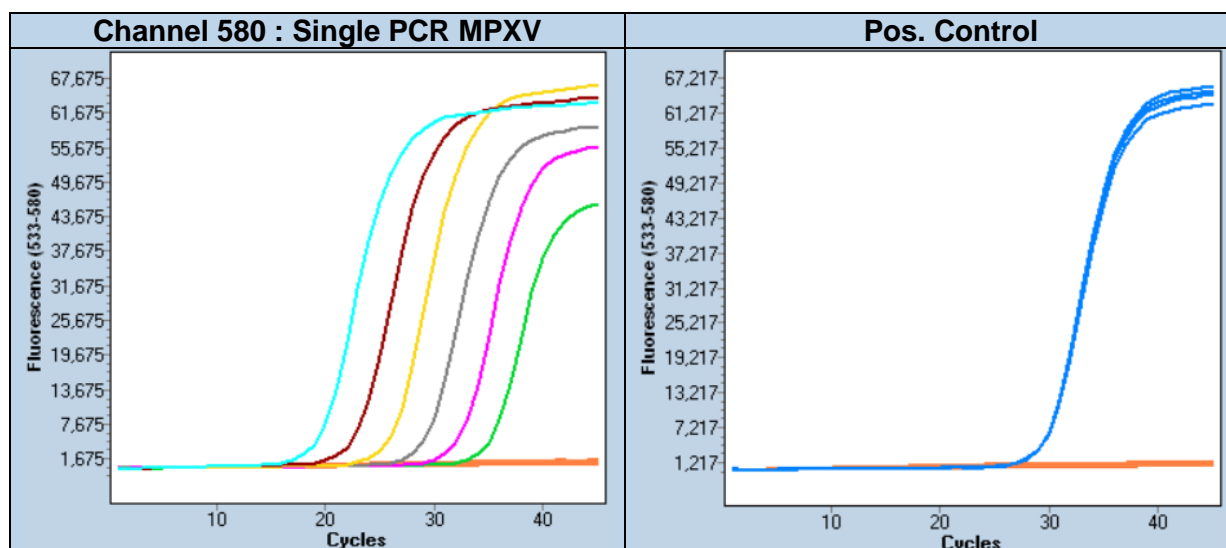
Mix gently, spin down and **transfer 15 µl** per well.

**Add 5 µl** of sample or control to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.

#### Start run

\* Pox is a DNA virus. No RT step required. The dried RT polymerase 90-9999 has been tested to work.

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



Dilution row 1E6 to 10 copies / reaction with LightCycler® Multiplex DNA Virus Master

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 580 channel. The negative control (NTC) must show no signal.

Channel 580 (sample)	Channel 660 Control Reaction	Channel 580 NTC Control	Result
No amplification	Detectable	Negative	Not detectable
Amplification Cp < 37 +	Not relevant	Negative	Monkeypox 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

Species-specific identification of variola, monkeypox, cowpox, and vaccinia viruses by multiplex real-time PCR assay, Shchelkunov (2011)

## 12. Multiplex PCR Compatibility

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

Multiplex PCR and Instrument Compatibility						480 II	z 480	LC96	LC2.0	Nano
500	530	580	610	640	660					
		Monkeypox				X	X	X	X	X
	Orthopox	Monkeypox			IC/EC	X	X	X		

Table 3

Recommended extraction controls are:


66-0901-96 PhHV  
 66-0905-96 MSTN  
 66-0907-96 RNase P  
 66-0913-96 Actin

Roche Cat.-No. 07 093 802 001  
 Roche Cat.-No. 07 225 253 001  
 Roche Cat.-No. 07 805 993 001

## 13. Version History

V220524	Release version	2022-05-24
V220526	Redefine Introduction, adding additional internal control options	2022-05-26
V220626	Probe R6G label. Kit can be used with polymerase 90-9999-96	2022-06-26
V220805	Additional information for use with 1-step RT polymerase 90-9999-96	2022-08-05
V220929	Dual target PCR	2022-09-29

**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° 5293 Expiry: YYYY-MM-DD							
Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp Measured							✓
Signal level Measured							✓
Negatives	10/10						✓
<b>Note:</b> 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 ( $\Delta$ Cp).							
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):							
Name1				Name2			

**TIB MOLBIOL** Syntheselabor GmbH | Eresburgstr. 22-23 | D-12103 Berlin | Germany  
 Tel. +49 30 78 79 94 55 | FAX +49 78 79 94 99 | dna@tib-molbiol.de | WWW.TIB-MOLBIOL.COM  
 HS code 3822 0000 | EORI DE 4806433 | Registry Court Berlin Charlottenburg HRB 93163 B

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