



### Instructions For Use

# LightMix<sup>®</sup> Modular Varicella-Zoster Virus

580

Cat.-No. 58-0132-96

Roche SAP 08 708 622 001-AU

Kit with reagents for 96 PCR reactions 20 µl for quantification of VZV [lyophilized]

## 1. Content, Storage and Expiry

- 1 Vial red cap 96 reactions VZV (lyophilized)
- 1 Vial black cap Positive Control (≈ Cp 30), lyophilized
- 1 Standard row with 10 to 10<sup>6</sup> 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<sup>®</sup> Multiplex DNA Master

Cat.-No. 07 339 585 001

## 3. Introduction

Varicella-Zoster Virus (VZV) is a member of the human herpesvirus family (HHV-3) causing chicken pox (varicella) and shingles (herpes zoster). VZV infections are medically significant, with approximately 4 million cases of varicella and 500,000 cases of zoster reported each year.

More than 90% of the population develop a varicella infection by the time they reach adolescence and virtually 100% of the population will have been infected by VZV by the age of 60. After the primary infection, the virus remains dormant - upon reactivation patients develop shingles, with an increasing risk with age. In immunosuppressed individuals VZV can cause severe and even fatal infections.

Common targets for PCR based tests are the thymidine kinase gene (orf26), glycoprotein gB (orf27), polymerase (orf28), the DNA binding protein (orf29), and the glycoprotein E gene (orf67). The virus can be found not only in blood but also in saliva and other body fluids.

## 4. Description

A 106 bp long fragment from the orf29 gene is amplified with specific primers and detected with a R6G labelled hydrolysis probe (580 channel).

## 5. Specification

This assay detects 10 genome equivalent copies or less per reaction (in vitro transcribed RNA).

## 6. Sample Material and Extraction

A typical specimen is a vesicle smear or swab, cerebrospinal fluid, whole blood or serum. 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

<b>Detection Format</b>	<b>Set Quant Factor 10, Max Integration time 1 sec</b>
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	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

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 **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 (≈ 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 concentrations, 10<sup>1</sup> to 10<sup>6</sup> copies per reaction (5 µl of reconstituted control). To prepare the standard row start with the standard of lowest concentration (first tube from the extended lip), use a 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 up and down ten times.

► **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	<b>Water</b> , PCR-grade (colorless cap, provided with the Roche Master kit)	5.5 µl
0.5 µl	<b>Reagent</b> mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	<b>Roche Master</b> (see Roche manual)	4.0 µl
<b>15.0 µl</b>	<b>Volume of Reaction Mix</b>	<b>10.0 µl</b>

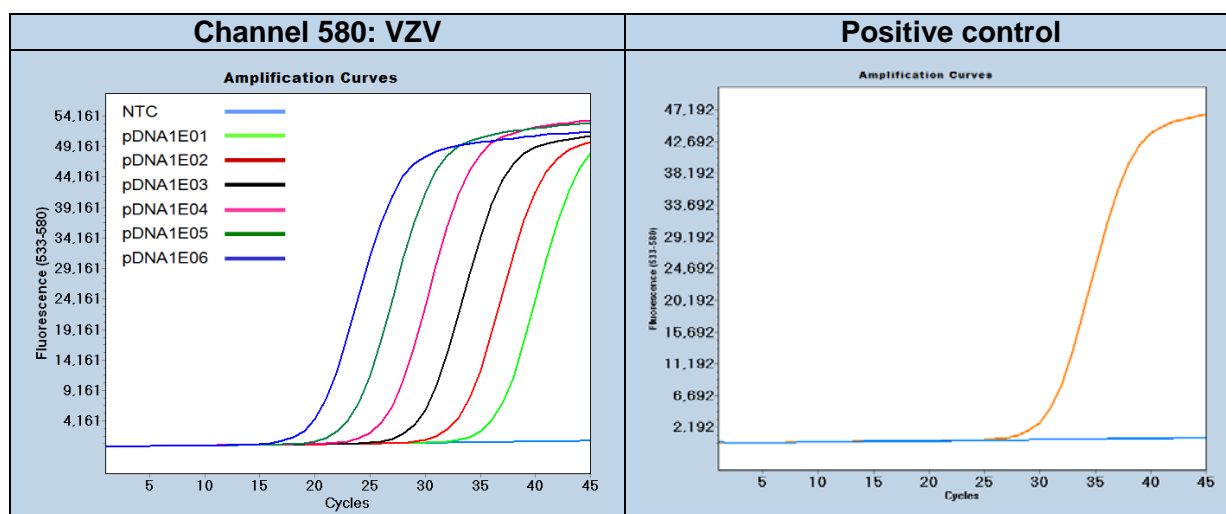
Table 2

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 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 < 39 <sup>+</sup>	Not relevant	Negative	VZV 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


This VZV assay can be combined with other LightMix<sup>®</sup> Modular assays, up to 6 targets per well including an internal control (IC) or an extraction control (e.g. PhHV or MSTN) as depicted below:

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	X	X	X		
		VZV			PhHV or MSTN	X	X			
	HSV-1	VZV		HSV-2		X				
CMV	HSV	VZV	EBV	HHV6		X				
CMV	HSV	VZV	Parvo B19	Toxoplasma		X				

Table 3

## 13. Version History

V151001	Release version	2015-10-01
V170830	Additional standard row for quantification	2017-08-30
V180424	CoA acceptance criteria updated. 1. Clarification storage conditions	2018-04-24
V180909	Editorial changes, Cp ranges updated, 8.2.2 Use Tris buffer	2018-11-19

Certificate of Analysis (CoA)							
Lot n°							
Expiry:							
<b>Dilution</b>	<b>1E6</b>	<b>1E5</b>	<b>1E4</b>	<b>PC</b>	<b>1E2</b>	<b>1E1</b>	<b>passed</b>
<b>Cp range</b>	19-21	23-25	26-28	30-32	33-35	36-38	
<b>Measured Signal level</b>	50-75						
<b>Measured</b>							
<b>Negatives</b>	<b>10/10</b>						✓
<p><b>Note:</b> 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 (<math>\Delta</math>Cp).</p>							
<p><b>QC Acceptance Date:</b></p>							
<p>We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.</p>							
<p><b>Name(s):</b></p>							

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