



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



Instructions For Use

LightMix[®] Modular Epstein-Barr-Virus (EBV)

610

Cat.-No. 61-0131-96

Roche SAP n° 00 000 000 000

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

1. Content, Storage and Expiry

- 1 Vial purple cap 96 reactions EBV (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
or Roche LightCycler[®] 480 Probes Master (no instructions included)

Cat.-No. 07 339 585 001
Cat.-No. 04 707 494 001

3. Introduction

The *Epstein-Barr Virus (EBV, Human Herpesvirus 4, HHV-4)* is a DNA virus from the Herpesvirus family. Most people become infected sometime during their lives but only a small fraction of infected people develop a disease. Children usually show no symptoms or indistinguishable from other mild illness. In the Northern hemisphere infections in adults can cause Infectious Mononucleosis (IM) known as 'Pfeiffer's disease' with several symptoms.

4. Description

A 78 bp long fragment from the viral BNRF1 gene is amplified with specific primers and detected with with a LC610 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 blood, tissue, or liquor (CSF). 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 610 Channel	Set Quant Factor 10, Max Integration time 2 sec
LightCycler® 480 Instrument:	558-610
LightCycler® 480 II Instrument:	533-610
cobas z 480 Analyzer (open channel):	540-610

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 Viral Nucleic Acid 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 **purple** 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

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

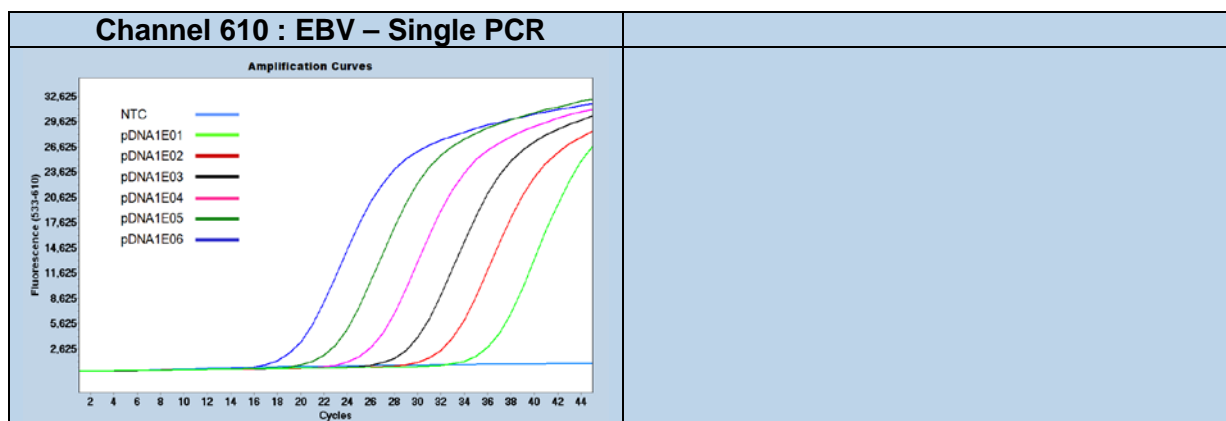
Table 2

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 510 (sample)	Channel 660 Control Reaction	Channel 510 NTC Control	Result
No amplification	Detectable	Negative	EBV not detectable
Amplification Cp < 39 ⁺	Not relevant	Negative	EBV 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

Development of a Real-Time Quantitative Assay for Detection of Epstein-Barr Virus. Hubert et al., 2000

12. Multiplex PCR Compatibility


This assay can be combined with other assays including an internal control (IC) or a spiked extraction control (for example PhHV) in the 660 channel 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					
			EBV			X	X	X		
CMV			EBV	HHV-6	PhHV	X	X			
	HSV	VZV	EBV	HHV-6		X	X			
CMV	HSV	VZV	EBV	HHV-6		X				

Table 3

13. Version History

V170808	Release version	2017-08-08
V190123	Editorial changes 8.2.2 Use Tris buffer	2019-03-21

Certificate of Analysis (CoA)							
Lot n° Expiry :							
Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range				29-31			
Measured Signal level				35-45			
Measured							
Negatives	10/10						✓
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 (Δ Cp).							
QC Acceptance Date:				YYYYMMDD			
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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