Instructions For Use

LightMix® Modular PhHV spiked Extraction Control 660

Cat.-No. 66-0901-96 Roche SAP n° 07 093 802 001

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

1. Content, Storage and Expiry

Storage at Arrival:

1 Vial green cap 96 reactions PhHV (lyophilized) Store cooled or at ambient temperature
1 Vial black cap containing the Extraction Control Target DNA **Do not freeze** the lyophilized reagents.

- Lyophilized kits are stable for at least 6 months (4°C to 25°C in the dark). See lot-specific expiry date.
- Dissolved reagents are stable for at least 2 weeks if stored protected from light and cooled (4°C).
- Dissolved reagents can be stored long-term at -20°C (within expiry). Avoid multiple freeze-thaw cycles.
- Dissolved Extraction Control Target must be stored at -20°C. Avoid multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex DNA Master	Cat.-No. 07 339 585 001
LightCycler® Multiplex RNA Virus Master	Cat.-No. 06 754 155 001
or Roche LightCycler® 480 Probes Master (no instructions included)	Cat.-No. 04 707 494 001

3. Introduction

PCR analysis of biological samples may occasionally result difficult due to problems related to the extraction process. In particular, false negative results in pathogen testing can be due to the presence of inhibitors, degraded target material or an unsuccessful extraction of nucleic acids. The presence of amplifiable nucleic acids can be verified by running an extraction control PCR.

This product is intended to be used as spiked extraction control and internal control to ensure for parameter-negative samples that extraction and PCR have been working and no inhibitors are present.

The amount of target spiked to the samples has to be adjusted to yield a Cp value in the range of 30. Note: With a MagNA Pure Compact the recovery rate for the EC target can be very low or even get lost.

4. Description

A 85 bp long fragment from the Phocine herpesvirus (PhHV) sequence target is amplified with specific primers and detected with a LC670 labeled hydrolysis probe.

5. Specification

This assay shall yield a Cp value of about 30 for analytical-PCR negative samples in multiplex PCR.

6. Sample Material and Extraction

Depends on the analytical PCR. See ModularDx Document ***Extraction Protocols***.

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).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

8. Instructions for Use

• Instrument programming see document ***ModularDx Programming***
• Color Compensation see instructions in ***40-0320 Universal Color Compensation Hexaplex***
• Pipetting instructions multiplex PCR see ***ModularDx Multiplex***

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 **Set Quant Factor 10, Max Integration time 3 sec**
LightCycler® 480 Instrument: 615-670
LightCycler® 480 II Instrument: 618-660
cobas z 480 Analyzer (open channel): 610-670

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

** 1-Step RT-PCR optional* . Table 1

8.2. Experimental Protocol

• **Sample material:** Use aqueous nucleic acid preparations (e.g. 'High Pure Viral Nucleic Acid Kit').

This is a control assay to verify that extraction worked. There is no need to include a 'negative control' (NTC) for the extraction control. Pathogen-assay negative controls (NTC) must be positive for the extraction control; negative control PCR results in pathogen-negative samples indicate an inhibition, extraction, or any other PCR or pipetting failure.

For an increased sensitivity use 10 µl sample per 20 µl reaction, in case that inhibition is likely to occur, e.g. extracts obtained from fecal samples, 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):

One reagent vial with a **green** cap contains all 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 Target Nucleic Acids (NA)

Add 1,200 µl RNase/DNase-free Tris buffer, PBS, or water to the **black** cap vial. Mix by pipetting up and down 10 times. If vortexing spin down to collect drops. The NA is not protected / encapsulated. Use of Tris buffer pH 8.0-8.5 increases the stability in solution. Store dissolved target NA frozen.

►**Add 10 µl** target NA to 200 µl of the sample to be extracted and extract immediately OR add during the lysis step. The amount of target NA is adjusted for a standard procedure with an extraction volume to 100 µl and a sample to PCR volume of 5-10 µl. The Cp value of the control reaction should be higher than 25. The amount of target NA may be varied to achieve a Cp value in the range of 27 to 33.

8.2.3. Preparation of the Reaction Mix

Multiply volumes by the number of reactions plus controls and one reserve and prepare in a cooled tube. The table contains the amounts for a duplex reaction for one analytical parameter (red) and the control:

For use with the Roche LightCycler® Multiplex RNA Virus / DNA Master		
for 5 µl extract	**Component**	**10 µl extract**
10.0 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.0 µl
0.5 µl	**PhHV Control Reaction** and	0.5 µl
0.5 µl	**PSR Reagent** mix additional assay(s) (Multiplex PCR)	0.5 µl
-	For each additional assay reduce the amount of water by 0.5 µl	-
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
0.1 µl	**Optional RT Enzyme** (RNA Master only) - reduce water by 0.1 µl	0.1 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 µl**

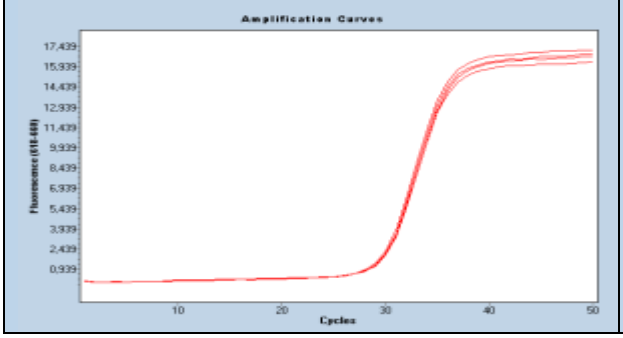
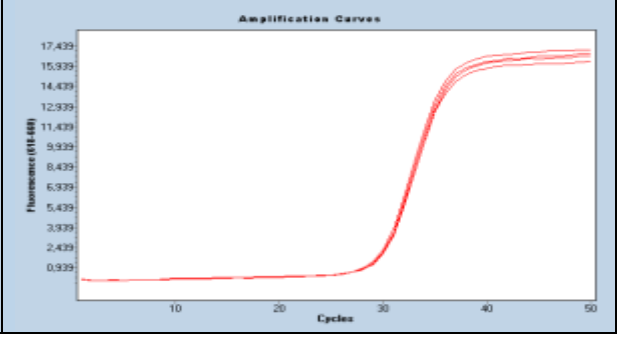
Table 2

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 660 : PhHV – Single PCR	Data obtained in Multiplex PCR
	

Here: 1E3 / 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 660 channel. The negative control (NTC) and negative samples must show a signal.

Other channel Analytical PCR	Channel 660 Control Reaction	Other channel NTC Control	Result
No amplification	**Amplification** +	Negative	Parameter - Negative
Amplification Cp < 37+	Not relevant	Negative	Parameter - Positive
No amplification	**Not detectable**	Not relevant	**PCR failure Repeat**
Amplification signal	Not relevant	Positive	Contamination Repeat

Notes: cobas z 480 Analyzer signal levels are about 50% compared to LightCycler® 480 II results.
+ Cp depends on the respective dilution during extraction copies.

11. References

Diagnosing herpesvirus infections by real-time amplification and rapid culture. van Doornum GJ, Guldemeester J, Osterhaus AD, Niesters HG. J Clin Microbiol. 2003 Feb;41(2):576-80

12. Multiplex PCR Compatibility

The PhHV internal control can be combined with up to three (LightCycler® 96), four (cobas z 480) or five (LightCycler® 480 system) analytical assays :

Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
	Assay 1				PhHV	X	X	X		
	Assay 1	Assay 2	Assay 3		PhHV	X	X	X		
	Assay 1	Assay 2	Assay 3	Assay 4	PhHV	X	X			
Assay 5	Assay 1	Assay 2	Assay 3	Assay 4	PhHV	X				

Table 3

13. Version History

V140404	Release version	2014-04-14
V140909	Editorial changes	2014-09-09
V150101	Editorial changes	2015-02-28
V150404	LightCycler® Nano removed from allowed instruments	2015-04-10
V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160313	1. Storage controls	2016-05-15

Certificate of Analysis (CoA)

Lot n°
Expiry :

TIB MOLBIOL

Dilution		**PSR**	**passed**
Cp range Measured		28-31	
Signal level Measured		10-20	
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 (ΔCp).

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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