



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



LightMix[®] Modular PhHV spiked Extraction Control

610

Cat.-No. 61-0901-96

Roche SAP n° 07 654 286 001

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

1. Content, Storage and Expiry

Storage at Arrival:

- 1 Vial purple cap 96 reactions PhHV (lyophilized)
 - 1 Vial black cap containing the Extraction Control Target DNA
- Store cooled or at ambient temperature
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 must be stored at -20°C. Avoid multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler[®] Multiplex DNA Master
LightCycler[®] Multiplex RNA Virus Master
or Roche LightCycler[®] 480 Probes Master (no instructions included)

Cat.-No. 07 339 585 001
Cat.-No. 06 754 155 001
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 LC610 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 610 Channel | Set Quant Factor 10, Max Integration time 3 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 |

* 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 **purple** 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 610 : 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 610 channel. The negative control (NTC) and negative samples must show a signal.

| Other channel than 610 Analytical PCR | Channel 610 Control Reaction | Other channel than 610 NTC Control | Result |
|---------------------------------------|------------------------------|------------------------------------|----------------------|
| No amplification | Amplification + | Negative | Parameter - Negative |
| Amplification signal | 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 (might have to be adjusted).

11. References

Diagnosing herpesvirus infections by RT-PCR and rapid culture. van Doornum et al. JCM. 2003 Feb;41(2):576-80

12. Multiplex PCR Compatibility

The PhHV extraction control assay can be combined with up to three (for LightCycler® 96 instruments), four (cobas z 480 analyze) or five (LightCycler® 480 systems) analytical assays :

Multiplex PCR and Instrument Compatibility
Color Compensation 40-0320 is mandatory for Multiplex PCR


| 500 | 530 | 580 | 610 | 640 | 660 |
|---------|---------|---------|------|---------|---------|
| | Assay 1 | | PhHV | | |
| | Assay 1 | Assay 2 | PhHV | | control |
| | Assay 1 | Assay 2 | PhHV | Assay 3 | control |
| Assay 4 | Assay 1 | Assay 2 | PhHV | Assay 3 | control |

| 480 II | z 480 | LC96 | LC2.0 | Nano |
|--------|-------|------|-------|------|
| X | X | X | X | X |
| X | X | X | | |
| X | X | | | |
| X | | | | |

Table 3

13. Version History

| | | |
|---------|--|------------|
| V150101 | Release version | 2015-02-28 |
| V150404 | Roche SAP number added | 2015-04-10 |
| V151001 | 2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition | 2015-10-01 |
| V160313 | 1. Storage controls, 8.1 LC480 filter settings | 2016-05-15 |

| Certificate of Analysis (CoA) | |  |
|--|--------------|---|
| Lot n° Expiry : | | |
| Dilution | PSR | passed |
| Cp range | 28-31 | |
| Measured Signal level | 35-45 | |
| 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> | | |
| QC Acceptance Date: | | YYYYMMDD |
| <p>We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.</p> | | |
| Name(s) : | | |

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