



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



Instructions For Use

LightMix[®] Modular Rhinovirus (HRV)

640

Cat.-No. 64-0123-96

Roche SAP n°07 730 420 001

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

1. Content, Storage and Expiry

- 1 Vial blue cap 96 reactions HRV (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.

- 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 positive controls must be stored at -20°C. Avoid multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler[®] Multiplex RNA Virus Master

Cat.-No. 06 754 155 001

3. Introduction

Rhinoviruses (HRV) have a positive-sense ssRNA genome and belong like Enterovirus to the family of the Picorna viruses. They are predominant cause of the common cold.

4. Description

A 141 bp fragment from the 5-UTR region is amplified with specific primers and detected with a LC640 labeled hydrolysis probe (640 channel).

5. Specification

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

6. Sample Material and Extraction

Typical clinical samples are nasopharyngeal, oropharyngeal or throat swabs, tracheal aspirates or bronchoalveolar lavage (BAL). 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 four program steps:

- 1: Reverse Transcription of the viral RNA
- 2: Denaturation: sample denaturation and enzyme activation
- 3: Cycling: PCR-amplification
- 4: Cooling: cooling the instrument

Detection Format 640 Channel	Set Quant Factor 10, Max Integration time 3 sec
LightCycler® 480 Instrument:	588-640
LightCycler® 480 II Instrument:	533-640
cobas z 480 Analyzer (open channel):	540-645

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

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 RNA with water.
- **Positive control:** Run a positive control - replace the template RNA with the provided Positive Control.

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 **blue** 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 Positive Control

Add 160 µl RNase/DNase-free Tris buffer or water to the vial with the **black** cap, for 10 µl sample add **320 µl**. Mix the solution by pipetting up and down 10 times. If vortexing spin down to collect the solution.

Notes: Opening of this vial may cause contaminations of the work-space (aerosol). Use of Tris buffer pH 8.0-8.5 increases the long-term stability in solution. Store dissolved controls frozen.

► **Use 5 µl** positive control (≈ 1,000 copies) for a 20 µl PCR reaction (or 10 µl if using 10 µl sample

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:

For use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	Component	10 µl extract
10.4 µl	Water , PCR-grade (colorless cap, provided with the Roche Master kit)	5.4 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
0.1 µl	RT Enzyme (see Roche manual)	0.1 µl
15.0 µl	Volume of Reaction Mix	10.0 µl

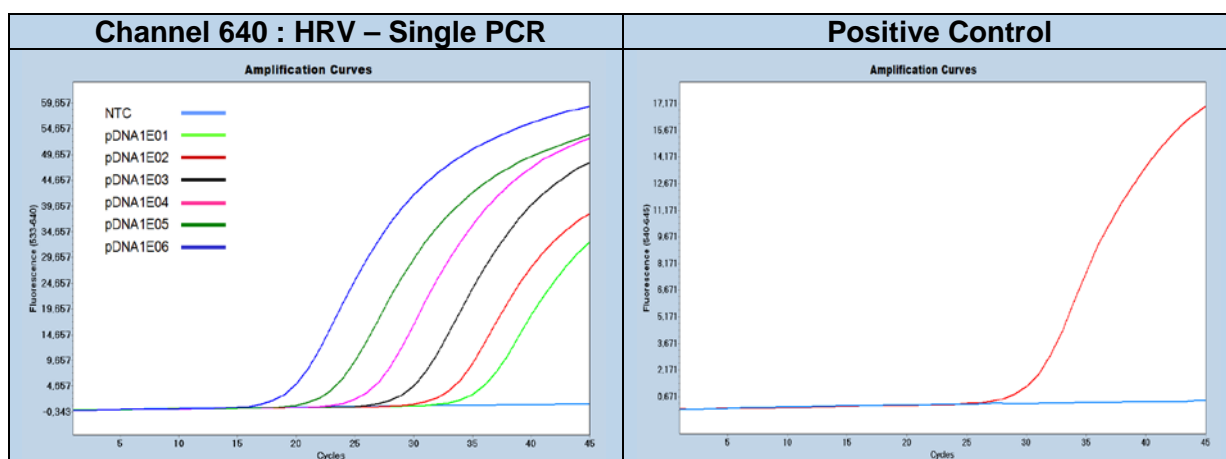
Table 2

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.

Add 5 µ (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 640 channel. The negative control (NTC) must show no signal.

Channel 640 (sample)	Channel 660 Control Reaction	Channel 640 NTC Control	Result
No amplification	Detectable	Negative	Not detectable
Amplification Cp < 37+	Not relevant	Negative	HRV 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.
+ Recommendation : Define the cut-off 2-4 cycles higher than observed for 10 copies.

11. References

Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. R.N. Gunson, T.C. Collins, W.F. Carman. J. Clin. Virology 33 (2005) 341–344

12. Multiplex PCR Compatibility Respiratory Virus Panel

This Rhinovirus assay can be combined with other assays up to 6plex reactions including an internal control (IC) or a spiked extraction control (for example EAV) as depicted below :

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


500	530	580	610	640	660
	hPeV	MPV	AdV	HRV	
	InfA	InfB	RSV-A/B	HRV	MSTN or PhHV or
H1 sw	InfA	InfB	RSV-A/B	HRV	EAV or
PIV-1,2,3,4	InfA	InfB	RSV-A/B	HRV	Roche RPC

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

Table 3

13. Version History

V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160116	Improved sensitivity in multiplex PCR. Not compatible with 50-0656-96	2016-01-16
V160313	Improved formulation . 1. Storage controls, 8.2.2 buffer, 8.2.3 wording	2016-05-21

Certificate of Analysis (CoA)							
Lot n° Expiry :							
Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	19-21	22-24	25-27	28-30	31-33	34-36	
Measured Signal level	40-50						
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 (ΔCp).							
QC Acceptance Date:							
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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