TIB MOLBIOL

Instructions For Use

LightMix® Modular panCoronavirus

500

Cat.-No. 50-0129-96 Roche SAP n° 07 766 254 001

Kit with reagents for 96 PCR x20 µl for detection of HKU1, OC43, 229E, NL63 and MERS [lyophilized]

1. Content, Storage and Expiry

Storage at Arrival:

1 Vial orange cap 96 reactions hCoV (lyophilized)
1 Vial black cap Positive Control (32,000 copies), Cp ~ 30

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 RNA Virus Master Cat.-No. 06 754 155 001

3. Introduction

Coronaviruses (CoV) are positive-stranded RNA viruses from the Coronaviridae family. The four common human pathogen strains 229E and NL63 from the Alpha group, and OC43 and HKU1 from the Beta group cause usally only a common cold, but the 2003 SARS-CoV pandemy with more than 800 fatal cases and the current MERS-CoV pandemy originating from Arabia made this virus family well worldwide known.

Coronaviruses are found preferentially in the lower respiratory system.

4. Description

90-115 bp long fragments from the Polyprotein gene from HKU1, OC43, 229E, NL63 and/or MERS CoV are amplified with specific primers and detected with Cyan500 labeled hydrolysis probes (500 channel). The test can not differentiate between the different viruses. The target for the MERS virus is different from the WHO-recommended target gene.

5. Specification

This assay detects 10-100 genome equivalent copies or less per reaction.

6. Sample Material and Extraction

Typical clinical samples are nasopharyngeal swabs, tracheal aspirates or bronchoalveolar lavage.
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 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 500 Channel **Set Quant Factor 10, Max Integration time 1 sec**
LightCycler® 480 Instrument: 450-500
LightCycler® 480 II Instrument: 440-488
cobas z 480 Analyzer (open channel): No filter combination for Cyan500 (opt. use FAM channel)

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 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 were 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 an orange 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 pH 8.0-8.5 to the vial with the black cap, if using 10 µl sample volume add **320 µl**. Mix the solution 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:

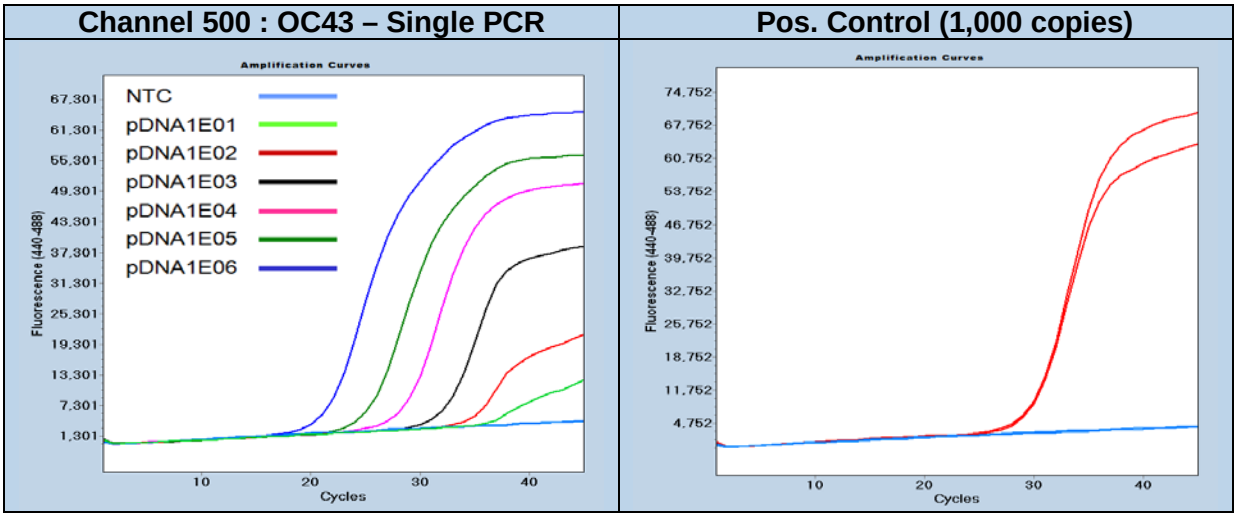
For use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
10.4 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.4 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control 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 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 Cyan500 channel. The negative control (NTC) must show no signal.

Channel 500 (sample)	Channel 660 Control Reaction	Channel 500 NTC Control	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 39+	Not relevant	**Negative**	**Corona Positive**
No amplification	**Not detectable**	Not relevant	**PCR failure Repeat**
Amplification signal	Not relevant	**Positive**	**Contamination Repeat**

Note: + Recommendation: Define the cut-off 2-4 cycles higher than observed for 10 copies

11. References

A pancoronavirus RT-PCR assay for detection of all known coronaviruses. Vijgen L, Moës E, Keyaerts E, Li S, Van Ranst M. Methods Mol Biol. 2008;454:3-12

12. Multiplex PCR Compatibility Respiratory Virus Panel

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

Coronavirus 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
Corona					MSTN, PhHV	X	X	X		
Corona	BocaV	MPV	AdV	HRV	or EAV or	X				
Corona	BocaV	MPV	AdV	EV	RNaseP or	X				
Corona	BocaV	MPV	AdV	hPeV	Roche RPC	X				

Table 3

13. Version History

V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160313	1. Storage of controls, 8.2.2 buffer, 8.2.3 wording	2016-05-21
V170830	hCoV 229E primer / probes adapted to increase sensitivity	2017-08-30
V180909	Editorial changes 8.2.2 Use Tris buffer	2018-11-01

Certificate of Analysis (CoA)

Lot n°
Expiry :

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Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range				28-30			
Measured							
Signal level				80-120			
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: YYYY-MM-DD

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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