



Instructions For Use

LightMix[®] Modular Wuhan CoV RdRP-gene

530

Cat.-No. 53-0777-96

Roche SAP n° 09 155 376 001

Kit with reagents for 96 PCR reactions 20 µl for detection of WH-Human_1 genomic RNA [lyophilized]

1. Content, Storage and Expiry

- 1 Vial yellow cap 96 reactions CoV (lyophilized)
- 1 Vial black cap Positive Control Cp ~ 30

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 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 usually only a common cold, but the 2003 SARS-CoV pandemic with more than 800 fatal cases and the current MERS-CoV pandemic originating from Arabia made this virus family well worldwide known.

The Wuhan CoV 2019 was described end of December 2019 after dozens of visitors of a seafood market selling all kind of animals developed severe pneumonia. Among 41 confirmed cases one person with serious underlying medical conditions died. One traveller in Bangkok reported. The genome sequence was published Jan 11th (Genbank acc. MN908947) and shows a high similarity to the SARS virus.

4. Description

A 100 bp long fragment from a conserved region of the RNA-dependent RNA polymerase (RdRP) gene is detected with FAM labeled hydrolysis probes (530 channel).

This assay will detect SARS and Wuhan 2019 CoV pneumonia virus as well as other bat-associated SARS-related viruses (Sarbecovirus); no cross reactivity with common human respiratory CoV NL63, 229E, HKU, OC43 or MERS

5. Specification

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

6. Sample Material and Extraction

Typical clinical samples are tracheal aspirates or bronchoalveolar lavage. Coronavirus affects the lower respiratory system; nasopharyngeal swabs / aspirate are not optimal.

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 Directive 67/548/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 530 Channel	Set Quant Factor 10, Max Integration time 1 sec
LightCycler® 480 Instrument:	483-533
LightCycler® 480 II Instrument:	465-510
cobas z 480 Analyzer (open channel):	465-510

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 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 **yellow** cap contains the primers and probe 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 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

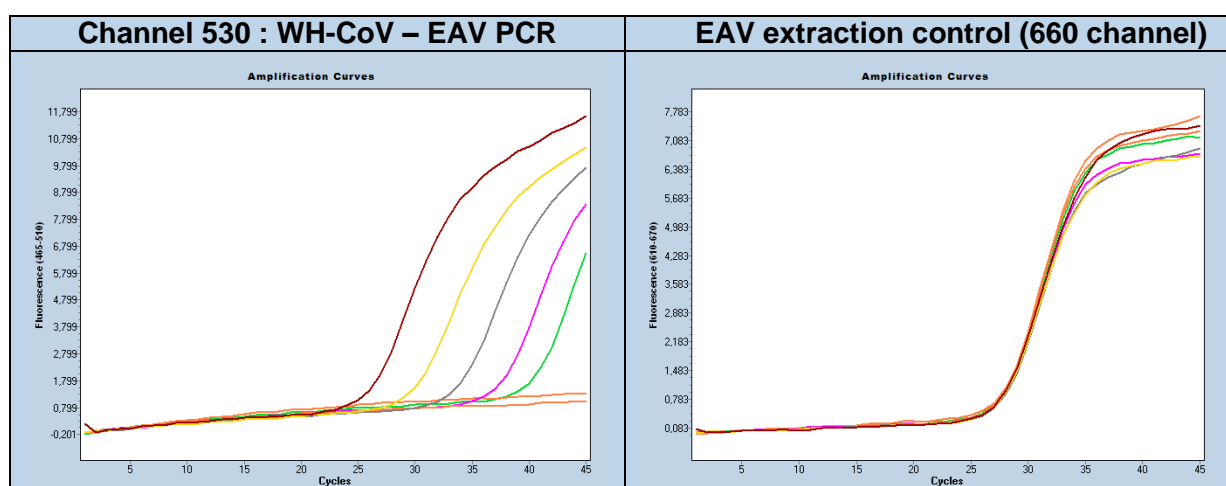
Table 2

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)



cobas z 480 data -dilution row of target

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 530 (sample)	Channel 660 Control Reaction	Channel 530 NTC Control	Result
No amplification	Detectable	Negative	Not detectable
Amplification Cp < 39 ⁺	Not relevant	Negative	WH-CoV 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

Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR. Wang et al., 2016
www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf?sfvrsn=d381fc88_2

12. Multiplex PCR Compatibility

This 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						480 II	z 480	LC96	LC2.0	Nano
Color Compensation 40-0320 is mandatory for Multiplex PCR										
500	530	580	610	640	660					
	WH-CoV	control				X	X	X	X	X
	WH-CoV				EAV	X	X	X		

Table 3

13. Version History

V200111 Release version

2020-01-11

Certificate of Analysis (CoA)							
Lot n° 4821 Expiry : YYYY-MM-DD							
Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range				29-31			
Measured							
Signal level				40-60			
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) :							

TIB MOLBIOL Syntheselabor GmbH | Eresburgstr. 22-23 | D-12103 Berlin | Germany
 Tel. +49 30 78 79 94 55 | FAX +49 78 79 94 99 | dna@tib-molbiol.de | WWW.TIB-MOLBIOL.COM
 Geschäftsführer (CEO): Olfert Landt | Register HRB 93163 B | Registergericht Berlin Charlottenburg

Distributed by Roche - www.lifescience.roche.com