



Instructions For Use

LightMix[®] Modular SARS-CoV (COVID19) N-gene

530

Cat.-No. 53-0775-96

Roche SAP n° 09 155 350 001

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

1. Content, Storage and Expiry

- 1 Vial yellow cap 96 reactions CoV (lyophilized)
- 1 Vial black cap RNA 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

The four common human Coronaviruses 229E, NL63, OC43 and HKU1 cause mild illness, like a common cold. The 2003 SARS pandemic and the MERS virus originating from Arabia made this virus family worldwide known. The 2019-nCoV pneumonia virus was reported end of December 2019 after dozens of visitors of a seafood market developed severe pneumonia (COVID-19). End of February 2020 there were 80,000 confirmed infections and 2,700 fatalities reported. The genome published Jan 11th (Genbank acc. MN908947) shows a high similarity to the SARS virus; the new name for the virus is SARS-CoV-2.

4. Description

A 128 bp long fragment from the N gene is detected with FAM labeled hydrolysis probes (530 channel). This assay will detect SARS and 2019-nCoV 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. The Cp values are approx 2 cycles delayed if compared to E gene.

The positive control contains 3 diagnostic targets E gene, N gene and RdRP (starting lot 48192008).

5. Specification

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

6. Sample Material and Extraction

Coronaviruses affect normally the lower respiratory system, but 2019-nCoV is found also in the nose, throat and the intestine. Typical clinical samples are tracheal aspirates, bronchoalveolar lavage, throat and nasopharyngeal swabs as well as stool samples.

For extraction protocols see Roche MagNA Pure or other manufacturer product 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

These Instruction for Use describes the use with Roche 480 instruments. BioRad CFX96, RotorGene LightCycler® 96 and SmartCycler give similar results. Other instruments not tested.

Roche 480 systems: Using 530+660 channels only (this kit) does not require a Color Compensation.

When run in combination further assays with other fluorophores (channels), a Color Compensation file must be applied. See instructions **40-0320-12 Color Compensation Hexaplex** (Roche 06296971001).

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

LightCycler® 480 Instrument:

LightCycler® 480 II Instrument:

cobas z 480 Analyzer (open channel):

Set Quant Factor 10, Max Integration time 1 sec

483-533

465-510

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

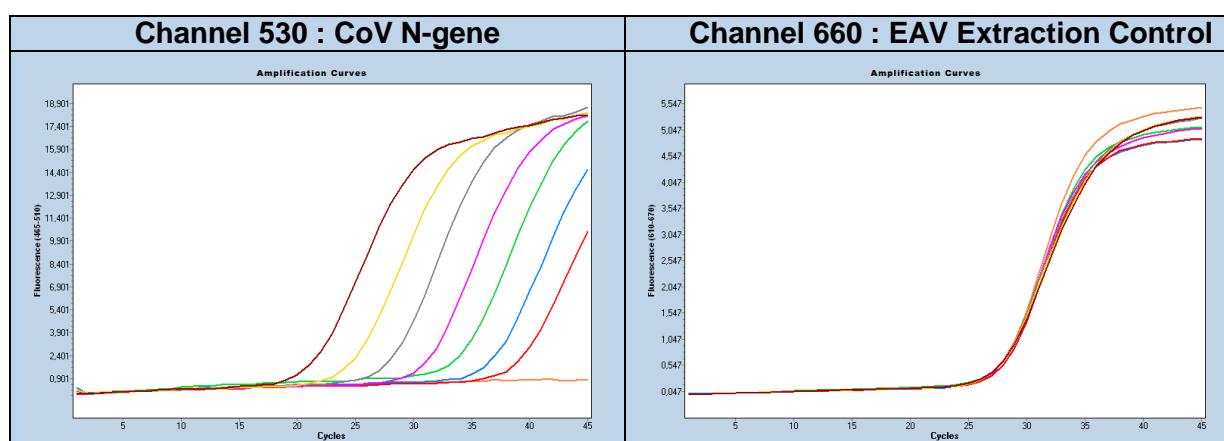
Table 2

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 < 37 ⁺	Not relevant	Negative	SarbecoV Positive
No amplification	Not detectable	Not relevant	PCR failure Repeat
Amplification signal	Not relevant	Positive	Contamination Repeat

+ Recommendation: Define the cut-off 1-2 cycles higher than observed Cp value for 10 copies.

11. References

Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Corman et al., 2020
<http://virological.org/t/initial-genome-release-of-novel-coronavirus/319>; Genbank acc. MN908947
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 EAV (66-0909-96 Roche 07374330001) as spiked extraction control or Internal Control (pre-extracted samples or extraction methods with bad efficiency for EAV: Use 0.5 µl of the solution per 20 µl PCR reaction. Currently no combinations with other ModularDx assays tested.

Multiplex PCR and Instrument Compatibility

Color Comp 40-0320 is mandatory only for Multiplex PCR using more channels

500	530	580	610	640	660
	SARS SARS				EAV


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

Table 3

13. Version History

V200111	Release version	2020-01-11
V200112	Disclaimer	2020-01-16
V200118	Product length 128 bp	2020-01-31
V200204	4. Positive control contains three targets (starting lot 48192008) 6. Specimen list extended 8. No ColorComp required for 530/660 10. Recommendation for setting the assay cut-off	2020-02-06
V200228	Product name changed (to comply with WHO recommendation)	2020-02-28

Note. EU / German Export Restrictions for this product (Dual Use Bioweapon Detection).
End-user-certificate may be required. End user will be reported to the National Authorities

Certificate of Analysis (CoA)							
Lot n° 4819 Expiry : YYYY-MM-DD							
Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	20-22	23-26	27-29	28-32	33-35	36-39	
Measured Signal level Measured	30-60						
Negatives	10/10						✓
<p>Notes: Cp (crossing point) values collected with pDNA in a single target PCR. Cp values may vary from instrument to instrument by up to 2 cycles, while the interval between two dilution steps is constant (ΔCp). In multiplex PCR Cp values are delayed. The cut-off is an interval based on the Cp value for the positive control; the cut-off in the CoA is a recommendation and must be set by the user. Fluorescence signal levels depend on instrument settings. Reported values are related to one reference instrument of the manufacturer. cobas z480 Analyzer signal levels are approx 50% compared to LC480 II results (more narrow filter bandwidth).</p>							
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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