TIB MOLBIOL

Instructions For Use

LightMix® Modular SARS-CoV-2 (COVID19) RdRP 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 SARS-CoV-2 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 pandemy 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 100 bp long fragment from a conserved region of the RNA-dependent RNA polymerase (RdRP) gene is detected with a SARS-CoV-2 specific FAM labeled hydrolysis probes (530 channel). No degenerate broad-range Sarbecovirus probe contained (probe P1 from the WHO protocol). Reagent has a different quencher and appears reddish instead of blue.

This assay will detect the Wuhan origin 2019-nCoV pneumonia virus but not all other SARS-like viruses. No cross reactivity with common human respiratory CoV NL63, 229E, HKU, OC43 or MERS.

The positive control contains 3 diagnostic targets E gene, N gene and RdRP (starting lot 48242009).

5. Specification

Sensitivity is 3.8 copies per reaction (95% CI: 2.7–7.6) (Corman et al. 2020). Lot release min 10 copies.
Note: The amplification is later compared to the E gene assay - all dilutions shifted 3 to 6 cycles.

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 otther 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	**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 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 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**

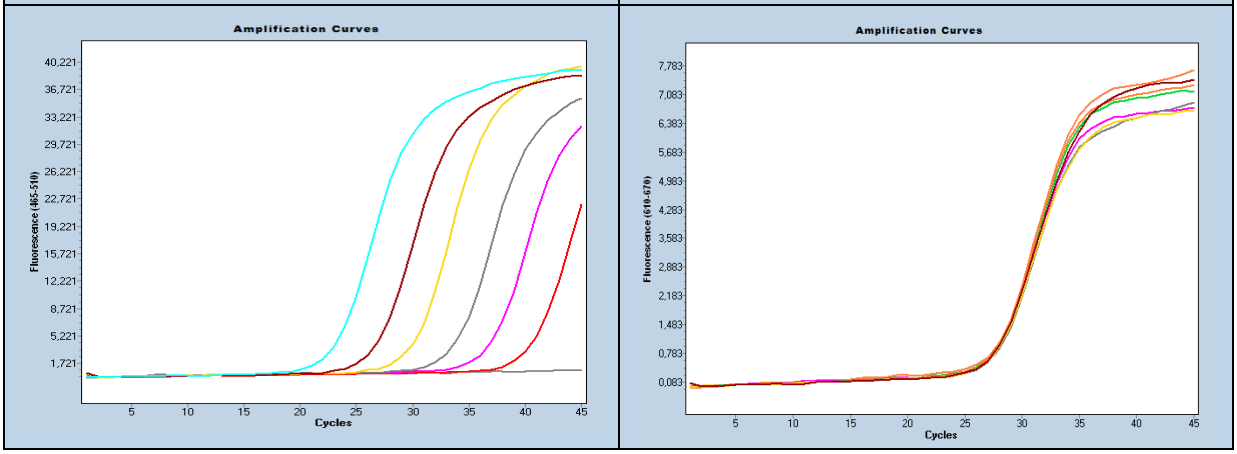
Table 2

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)

Channel 530 : CoV RdRP	Channel 660 : EAV Extraction Control



LC480II data -dilution row of plasmid target 1E6 to 10 copies 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**	**SARS-CoV-2 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

V200112	Release Version / Export Disclaimer	2020-01-16
V200118	Probe swabed to the complement strand (improved perfomance)	2020-01-18
V200204	**4.** Positive control contains three targets (starting lot 48242009)	2020-02-06
	6. Specimen list extended **8.** No ColorComp required for 530/660	
	10. Recommendation for setting the assay cut-off	
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° **4824**

Expiry :

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Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	22-24	25-28	29-31	28-32	35-38	38-40	
Measured							✓
Signal level				30-60			
Measured							✓
Negatives	10/10						✓

***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).

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) :

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