Instructions for research-use. For *in vitro* use only.

Instructions For Use

LightMix® SarbecoV E-gene plus EAV control 530/660

Cat.-No. 40-0776-96 Roche SAP n° 09 164 154 001

Kit with reagents for 96 PCR reactions 20 µl for detection of WH-Human_1 genomic RNA [lyophilized]
Mixture of ModularDx kits 53-0776 Wuhan E-gene and 66-0909 EAV - **No repeat evaluation required**.

1. Content, Storage and Expiry

1 Vial yellow cap 96 reactions CoV (lyophilized)
1 Vial black cap RNA Positive Control Cp ~ 30
1 Vial white cap EAV extraction control target

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 Wuhan 2019-nCoV was reported first end of December 2019 after dozens of visitors of a seafood market developed severe pneumonia. Early February 2020 there were 27,000 confirmed infections and 560 fatalities. The genome published Jan 11th (Genbank acc. MN908947) shows a high similarity to the SARS virus.

4. Description

A 76 bp long fragment from the E gene is amplified with specific primers and detected with a FAM label hydrolysis probe. The assay detects SARS and 2019-nCoV pneumonia virus (bat-associated SARS-related Sarbecovirus); no cross reactivity with CoV NL63, 229E, HKU, OC43, or MERS.

Control reaction: 70 bp fragment from Equine Arteritis Virus detected with an Atto647 labeled probe.

The RNA positive control contains all diagnostic targets E gene, N gene and RdRP (Corman, 2020).

5. Specification

Sensitivity is 5.2 copies per reaction (95% CI: 3.7–9.6) (Corman et al. 2020). Lot release min 10 copies.

6. Sample Material and Extraction

Coronaviruses affect normally the lower respiratory system, but 2019-nCOV is found also in the nose, throut and the intestine. Typical clinical samples are tracheal aspirates, bronchoalveolar lavage, throut 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 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 in the ***Roche 06296971001 Universal Color Compensation Hexaplex.***

8.1. Programming Roche 480 Instruments

See the Instrument operator's manual for details. Start programming before preparing the solutions.

Detection Format Dual Channel	**Set Quant Fact. 10, Max Int. time 1 sec 530, 3 sec 660**	
LightCycler® 480 Instrument:	483-533	615-670
LightCycler® 480 II Instrument:	465-510	618-660
cobas z 480 Analyzer (open channel):	465-510	610-670

FastCycle program 50 min. ModularDx standard program yields same results. The protocol has 4 steps:

Program Step:	***RT Step***	***Denaturation***	***Cycling***		***Cooling***
Parameter					
Analysis Mode	***None***	None	Quantification mode		None
Cycles	*1*	1	40		1
Target [°C]	55	95	95	60	40
Hold [hh:mm:ss]	00:03:00	00:00:30	00:00:03	00:00:12	00:00:10
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	2.0
Acquisition Mode	None	None	None	**Single**	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 increased sensitivity use 10 µl extract per 20 µl reaction; (384 well plates use 5 µl per 10 µl reaction).

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+ 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. Store frozen.

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 Extraction Target Nucleic Acid (RNA)

Add 1,200 µl RNase/DNase-free 10 mM Tris buffer pH 8 - 8.5 to the vial with the **white** cap. Mix by pipetting up and down 10 times. If vortexing spin down. Store frozen.

►**Add 10 µl** target NA to 200 µl of the sample to be extracted and extract immediately OR add during the lysis step. The amount of target NA is adjusted for a standard procedure with an extraction volume to 100 µl and a sample to PCR volume of 5-10 µl. The Cp value of the control reaction should be later than 25. The amount of target NA may be varied to achieve a Cp value in the range of 27 to 33.

Optional: For using EAV as Internal PCR Control (for example pre-extracted samples or extraction methods with a bad efficiency for EAV) use 0.5 µl of the solution per 20 µl PCR reaction.

8.2.4. 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
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**

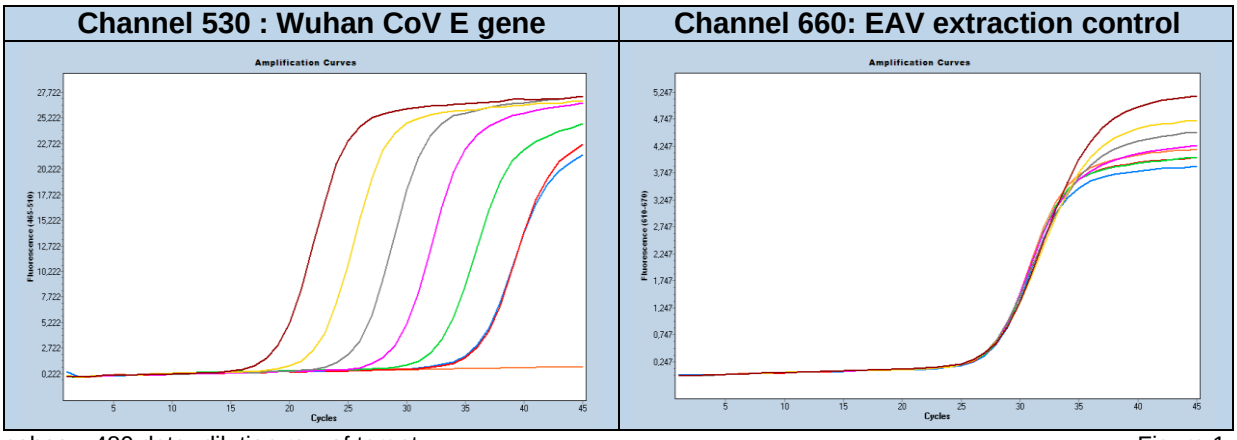
Table 2

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

Note: Using other manufacturer's 1-step RT PCR reagent will give similar results but needs to be verified.

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 < 36+	**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 includes EAV as spiked extraction control (may be used as Internal Control only; see 8.2.3). Currently no combinations with other ModularDx assays tested.

Multiplex PCR and Instrument Compatibility

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

500	530	580	610	640	660
	WH-CoV				EAV

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

Table 3

13. Version History

V200204	**4.** Pos control 3 targets **6.** Specimen list **8.** No ColorComp required **8.1** FastCycle PCR (optional). **8.2.3**. Internal PCR Control instead of Extraction Control **10.** Recommendation for the diagnostic cut off	2020-02-04

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° **4827**

Expiry : **YYYY-MM-DD**

TIB MOLBIOL

Dilution	1E6	1E5	1E4	PC	1E2	1E1	Ctrl.660	passed
Cp range	18-20	22-23	24-27	28-31	31-33	34-36	27-33	
Measured								
Signal level				30-60			10-25	
Measured								
Negatives	20/20							✓

***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: 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) :

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