Instructions For Use

LightMix® Modular SARS and Wuhan CoV E-gene
Cat.-No. 53-0776-96
Roche SAP n° 09 155 368 001
Kit with reagents for 96 PCR reactions 20 µl for detection of WH-Human_1 genomic RNA [lyophilized]

1. Content, Storage and Expiry

**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 pandemy with more than 800 fatal cases and the current MERS-CoV pandemy 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 76 bp long fragment from a conserved region in the E 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RT Step</th>
<th>Denaturation</th>
<th>Cycling</th>
<th>Cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis Mode</td>
<td>None</td>
<td>None</td>
<td>Quantification mode</td>
<td>None</td>
</tr>
<tr>
<td>Cycles</td>
<td>1</td>
<td>1</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Target [°C]</td>
<td>55</td>
<td>95</td>
<td>95</td>
<td>60 *</td>
</tr>
<tr>
<td>Hold [hh:mm:ss]</td>
<td>00:05:00</td>
<td>00:05:00</td>
<td>00:00:05</td>
<td>00:00:15</td>
</tr>
<tr>
<td>Ramp Rate [°C/s] 96</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
<td>2.2</td>
</tr>
<tr>
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<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Acquisition Mode</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Single</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Component</th>
<th>for 5 µl extract</th>
<th>10 µl extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, PCR-grade (colorless cap, provided with the Roche Master kit)</td>
<td>10.4 µl</td>
<td>5.4 µl</td>
</tr>
<tr>
<td>Reagent mix (parameter specific reagents containing primers and probes)</td>
<td>0.5 µl</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>Control Reaction and additional assays (Multiplex PCR)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Roche Master (see Roche manual)</td>
<td>4.0 µl</td>
<td>4.0 µl</td>
</tr>
<tr>
<td>RT Enzyme (see Roche manual)</td>
<td>0.1 µl</td>
<td>0.1 µl</td>
</tr>
<tr>
<td>Volume of Reaction Mix</td>
<td>15.0 µl</td>
<td>10.0 µl</td>
</tr>
</tbody>
</table>

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)

![Amplification Curves Channel 530 and 660](image)

Table 2

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.

<table>
<thead>
<tr>
<th>Channel 530 (sample)</th>
<th>Channel 660 Control Reaction</th>
<th>Channel 530 NTC Control</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amplification</td>
<td>Detectable</td>
<td>Negative</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Amplification Cp &lt; 39°</td>
<td>Not relevant</td>
<td>Negative</td>
<td>WH-CoV Positive</td>
</tr>
<tr>
<td>No amplification</td>
<td>Not detectable</td>
<td>Not relevant</td>
<td>PCR failure Repeat</td>
</tr>
<tr>
<td>Amplification signal</td>
<td>Not relevant</td>
<td>Positive</td>
<td>Contamination Repeat</td>
</tr>
</tbody>
</table>

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

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**
Color Compensation 40-0320 is mandatory for Multiplex PCR

<table>
<thead>
<tr>
<th></th>
<th>500</th>
<th>530</th>
<th>580</th>
<th>610</th>
<th>640</th>
<th>660</th>
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<tbody>
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<tr>
<td>control</td>
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<td>WH-CoV</td>
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<tr>
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</table>

Table 3

13. Version History

V200111 Release version 2020-01-11

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**Certificate of Analysis (CoA)**
Lot n° 4820
Expiry : YYYY-MM-DD

<table>
<thead>
<tr>
<th>Dilution</th>
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<th>PC</th>
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<th>1E1</th>
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<tbody>
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<tr>
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<td></td>
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</table>

*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: 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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