Coronavirus (SARS-CoV-2) RT-qPCR detection kit
[N1 and RNAse P genes-based assay]

Cat. No: E01595 (100 rxns)

Introduction

A novel coronavirus, currently termed COVID-19 (2019-nCoV or SARS-CoV-2), was announced as the etiological agent of cases of ongoing pneumonia outbreak in Wuhan City (China). This kit describes the use of real time RT-PCR for the in vitro detection of SARS-CoV-2 in respiratory specimens (sputum; nasopharyngeal, oropharyngeal aspirates, washes or swabs; tracheal aspirates).

One-Step RT-qPCR allows efficient cDNA synthesis and Real-Time PCR in a single tube. The kit includes a RT-qPCR Master Mix supplied in a 4X concentration and a vial with Ribonuclease Inhibitor.

Also, the kit contains one set of primers and fluorescent probe designed according to CDC (see below for reference) to target region of the virus nucleocapsid (N) gene and to detect the human RNase P used as a control to assess specimen quality. The probes are readout in different channels. Coronavirus SARS-CoV-2 RNA target is amplified and detected in the FAM channel. Human RNase P target is amplified and detected in the HEX, VIC or JOE channel (depending on the equipment used).

The assay does not include a positive control (PTC). A PTC is needed to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a negative control reaction should be included every time the kit is used.

Each kit contains: 100 reactions of 20 µL/each

- 4X RT-qPCR Mix (1 vial, lyophilized)
- Reconstitution Solution (1 vial)
- Ribonuclease inhibitor (1 vial)
- Primer/ Probe Mix (1 vial)

Storage

SARS-CoV-2 RT-qPCR Kit is shipped on gel pack. The Kit should be stored at -20°C upon receipt. Avoid repeated freezing and thawing. Maintain cold when thawed.

PCR Primers&Probe Mix must be kept in the dark. Mix gently and aliquot in different tubes. Store aliquots of primers&probe sets at -20ºC.

4X RT-qPCR Master Mix RECONSTITUTION

1) Transfer 500 µL of the vial Reconstitution Solution to the vial 4X RT-qPCR Master Mix.
2) Mix well pipetting up and down slowly – the lyophilisate will dissolve within seconds.
3) Once the 4X RT-qPCR Master Mix has been reconstituted, store it at -20ºC.

PCR Primer/ Probe Mix is referenced in publicly available 2019-nCoV real-time PCR protocols for Emergency Use Authorization (information can be found at CDC [https://www.fda.gov/media/134922/download]).
**Technical Recommendations:**
- Extraction of the RNA from sample is mandatory before use in real time RT-PCR.
- The quality of the extracted RNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology.
- When performing RNA extraction, an internal extraction control should be included.

**PROCEDURE**

1. **Thaw kit components on ice. Mix each solution well.**

2. **Set up the following reaction mixture for each PCR Primer/ probe Mix.**
   The following protocol is recommended for a 20 µL reaction volume:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume reaction *</th>
</tr>
</thead>
<tbody>
<tr>
<td>4X qPCR Master Mix</td>
<td>5 µL</td>
</tr>
<tr>
<td>Ribonuclease Inhibitor</td>
<td>0,5 µL</td>
</tr>
<tr>
<td>PCR Primer/ Probe Mix</td>
<td>5 µL</td>
</tr>
<tr>
<td>Water for Molecular Biol</td>
<td>4,5 µL</td>
</tr>
</tbody>
</table>

   *Multiply all values according to experimental needs plus a 10%.

3. **After mixing reagents above, distribute 15 µL into the number of wells required for your testing.**
   Include 1 well for the NTC and 1 well for the PTC (not supplied) for each run.

4. **Add 5 µL of RNA extracted from each sample. Please include a negative template control (NTC) with 8 µL water for Molecular Biology and PTC (not supplied) in different wells.**
   NTC consists of using nuclease-free water in the RT-qPCR reactions instead of RNA.
   *The quality of the test depends on the quality of the RNA sample. Unsuitable collection, storage and/or transport of specimens may give false negative results.*

5. **Centrifuge briefly to collect the contents of the wells at the bottom. Protect from extended light exposure or elevated temperatures before cycling.**

6. **Program the appropriate PCR cycling protocol on your real-time PCR instrument**
   **Suggested Thermal Cycling Conditions**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>42°C</td>
<td>30 min</td>
<td>1</td>
</tr>
<tr>
<td>Initial activation</td>
<td>95°C</td>
<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>15 sec</td>
<td>45</td>
</tr>
<tr>
<td>Annealing and extension*</td>
<td>55°C</td>
<td>30 sec</td>
<td></td>
</tr>
</tbody>
</table>

   * Acquisition must be performed at the end of this stage
6. Select the fluorescent channel (FAM/HEX) of instrument for testing.

2019-nCov = FAM (465-510)
RNase P = VIC / HEX / JOE (533-580)

7. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

ANALYSIS OF RESULTS

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data.

Controls

✓ Non-Template Control (NTC) should be negative and must not give Ct value of less than 40 in FAM channel. If NTC reaction is positive, sample contamination has been occurred.

✓ Positive Template Control (PTC) should be positive and must give a Ct of less than 40 in FAM channel. If this result is not obtained, repeat the assay implementing corrective actions for failed reactions.

✓ RNase P Extraction Control should be positive and exhibited an expected Ct value for HEX, VIC or JOE channel. If N gene-based reaction and RNase P reaction are negative for the sample, the result should be considered invalid. But if the N1 reactions are positive, even in the absence of a positive RNase P, the result should be considered valid.

Samples

When all controls exhibit the expected performance then the samples could be positive, negative or suspicious:

✓ Positive: If Ct ≤ 40 in FAM channel.

✓ Negative: If there is no Ct value in the FAM channel.

✓ Suspicious samples: Samples with Ct value greater than 38, it is recommended to re-extract RNA for RT-PCR. If the result is still less than 40, the sample can be reported as positive, otherwise it is negative.

Notes:

• False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.

• As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
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.

Product use limitation

In response to the new coronavirus (2019-nCoV) emergency, this kit is intended to facilitate and support research. This kit is a method of assistant diagnosis for research use only and cannot be used as a basis for confirming or excluding cases.

Research Use Only (RUO).