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Coronavirus (SARS-CoV-2) PCR Detection Kit

03/20

(Catalog # K1460-100 Rxns; Store at -20°C)

I. Introduction:

BioVision's Coronavirus (SARS-CoV-2) PCR-Detection Kit is used for the *in vitro* detection of SARS-CoV-2 in respiratory specimens using Real-Time (RT-PCR). The coronavirus SARS-CoV-2 was announced as the etiological agent of cases of ongoing pneumonia outbreak in Wuhan City, China. BioVision's Coronavirus Detection Kit allows efficient cDNA synthesis and RT-PCR in a single tube. This kit includes a qPCR master mix that contains all the reagents supplied in a 2X concentration to perform the RT-PCR. A separate Reverse Transcription mix that comprises a balanced mixture of both Reverse Transcriptase and RNase Inhibitor is included in the kit. In addition, the kit contains one set of primers and fluorescent probes to differentiate between SARS-CoV-2 and SARS-CoV (or bat SARS related CoV). The probes are read in different channels. Coronavirus SARS-CoV-2 RNA targets are amplified and detected in the FAM channel, Coronavirus SARS-CoV-2, SARS-CoV (or bat SARS related CoV) RNA targets are amplified and detected in the HEX, VIC or JOE channel (depending on the equipment used). The kit can be used to detect SARS-CoV-2 in respiratory specimens such as sputum, nasopharyngeal, oropharyngeal aspirates, washes or swabs and tracheal aspirates. BioVision offers the PCR Detection Kit to facilitate research on Coronavirus and improve patient treatment.

II. Application:

- An ideal tool to detect SARS-CoV-2 by RT-PCR method

III. Key Features:

- Rapid, reliable amplification and detection
- Inclusivity: SARS like Coronavirus and **specific detection of SARS-CoV-2**
- **Dual Color** Multiplex Assay Format
- Includes positive and negative controls

III. Sample Types:

- Respiratory specimens such as sputum, nasopharyngeal, oropharyngeal aspirates, washes or swabs
- Tracheal aspirates

V. Kit Contents:

Components	K1460-100 (100 Rxns)	Part Number
2X qPCR Master Mix	1 vial	K1460-XX-1
Reverse Transcription Mix	1 vial	K1460-XX-2
PCR Primer/ Probe set	1 vial	K1460-XX-3
Rehydration Buffer	1 vial	K1460-XX-4
COVID-19 Positive control (PTC)	1 vial	K1460-XX-5
Non-Template Negative Control (NTC)	1 vial	K1460-XX-6

VI. User Supplied Reagents and Equipment:

- qPCR Thermal Cycler
- PCR tubes

VII. Shipping and Storage Conditions:

The kit is shipped in gel packs. The kit should be stored at -20°C upon arrival. Avoid repeated freezing and thawing. Keep the reagents on ice when thawed. Avoid prolonged exposure to light. The kit reagents will be stable for 12 months, if stored as recommended.

VIII. Reagent Preparation and Storage Conditions:

PCR Primers/ Probe Set must be rehydrated in the dark. Mix gently and aliquot in different tubes. Store the aliquots at -20°C. **PCR Primers & Probe set is referenced in publicly available SARS-CoV-2 RT-PCR protocols (information can be found on the WHO website).**

IX. PCR Detection Assay Protocol:

1. Thaw all kit components on ice. Mix each solution well. The following protocol is recommended for a 20 µl reaction volume.
2. Set up the following reaction mixture.

	Volume/reaction*
2X qPCR Master Mix	10 µl
Reverse Transcription Mix	1 µl
PCR Primer/Probe set	4 µl

* Multiply all numbers according to experimental requirements.

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3. After mixing the above reagents, add 15 µl into the number of wells required for your testing (include 1 well for the NTC and 1 well for the PTC).
4. Add 5 µl of RNA extracted from each sample, NTC and PTC in different wells and close them with the caps provided. 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. Program the appropriate PCR cycling protocol on your real-time PCR instrument.

Suggested thermal cycling conditions are given below:

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	10 min	1
Initial activation	95°C	3 min	1
Denaturation	95°C	15 sec	45
Annealing and extension	58°C*	30 sec	

*Acquisition must be performed at the end of this stage.

6. Select the fluorescent channel (FAM/HEX) on the instrument for testing.
 - (a) SARS-CoV-2 = FAM (465 nm - 510 nm)
 - (b) SARS-CoV, SARS-CoV (or bat SARS related CoV) = VIC / HEX / JOE (533 nm - 580 nm)
7. **Analysis of results:**

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

 - (a) Controls
 - Positive Template Control (PTC) should be positive and exhibit an expected C_t value for each channel included in the kit. If these results are not obtained, repeat the assay implementing corrective actions for failed reactions. The assay must be positive in both FAM and HEX channel for SARS-CoV-2 detection.
 - Non-Template Control (NTC) should be negative and not exhibit C_t value in FAM channel and HEX channel. If NTC reaction is positive, sample contamination has occurred.
 - (b) Samples: When all controls exhibit the expected performance then the samples could be positive, negative or suspicious
 - Positive: If C_t ≤ 35 in both FAM and HEX channel.
 - Negative: If there is no C_t value in any of the FAM channel and HEX channel.
 - Suspicious samples: If there is no C_t value in any of the FAM and HEX channel, it is recommended to re-extract the RNA. If the result is the same, the sample can be reported as negative. For samples with C_t value > 35, it is recommended to re-extract RNA for RT-PCR. If the result is still < 40, the sample can be reported as positive, otherwise it is negative.

X. Related Products:

BioVision Product Name	Cat. No.	Sizes
PCR-Salmonella Detection Kit	K1447	96 Rxns
PCR-Salmonella-Listeria Detection Kit	K1448	96 Rxns
PCR-Listeria monocytogenes Detection Kit	K1449	96 Rxns
PCR-Legionella spp Detection Kit	K1450	96 Rxns
PCR-Legionella spp Plus Detection Kit	K1451	96 Rxns
PCR-STEC Detection Kit	K1452	96 Rxns
PCR-Campylobacter Detection Kit	K1453	96 Rxns

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