

FOR RESEARCH USE ONLY!

Coronavirus Rapid RT-qPCR Detection Kit

03/20

(Catalog # K1461-100 Rxns; Store at -20°C)

I. Introduction:

Coronavirus rapid RT-qPCR Detection Kit is used for the detection of SARS-CoV-2 in respiratory specimens using Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR). It allows the efficient cDNA synthesis and qPCR in a single tube. This kit includes a 2X RT-qPCR Mastermix that contains all the reagents supplied in a 2X concentration to perform the qPCR. A separate RT-qPCR Enzyme Mix for cDNA synthesis is included the kit. Additionally, the kit contains 2 vials of primers and probes specific for the ORF1ab and N target. The PCR Detection Kit will facilitate the Coronavirus research and improve patient treatment.

II. Application:

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

III. Key Features:

- Reliable and Ready-to-use
- Results ready in less than 2 hr
- Highly specific for the ORF1ab and N target markers recommended by WHO and US CDC
- Includes a **Positive Control**
- Compatible with standard RT-qPCR machines (Bio-Rad CFX96, QuantStudio's 7 Flex system)

IV. Sample Types:

Human respiratory specimens, sputum and naso/oropharyngeal swab samples

V. Kit Contents:

Components	K1461-100 (100 Rxns)	Part Number
2X RT-qPCR Master Mix	1.25 ml	K1461-XX-1
COVID-19 Primers	100 µl	K1461-XX-2
COVID-19 Probes	100 µl	K1461-XX-3
Positive Control Template	10 µl	K1461-XX-4
RT-qPCR Enzyme Mix	40 µl	K1461-XX-5
Nuclease-free Water	1 ml	K1461-XX-6

VI. User Supplied Reagents and Equipment:

- qPCR Thermal Cycler
- PCR tubes
- Nuclease-free Water

VII. Shipping and Storage Conditions:

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.

VIII. Assay Protocol:

- Thaw the following reagents on ice: COVID-19 Primers, COVID-19 Probes, 2X RT-qPCR MasterMix, and RT-qPCR Enzyme Mix. Mix individual components before use and assemble reaction on ice. Do not add more than one RNA sample into a single qPCR tube. Start with 5 pg -1 µg *RNA sample per 20 µl reaction. We recommend using a reaction volume of 20 µl.

Note: *RNA sample can be prepared using BioVision Cat# M1461

Components	Sample	Positive Control	Negative Control
RNA Sample	As required	As required	As required
COVID-19 Primers	1 µl	1 µl	1 µl
COVID-19 Probes	1 µl	1 µl	1 µl
2X RT-qPCR MasterMix	10 µl	10 µl	10 µl
RT-qPCR Enzyme Mix	0.4 µl	0.4 µl	0.4 µl
Positive Control Template	-	1 µl	-
Nuclease-free Water	Make up to 20 µl	Make up to 20 µl	Make up to 20 µl

- Use the Nuclease-free Water for Negative Control and the Positive Control Template for setting up the Positive Control.
- Gently mix the reaction components. Briefly centrifuge. Transfer the reaction setup to qPCR machine.

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Standard RT-qPCR Cycling Condition:

Transfer the reaction setup into qPCR machine and set up the following cycling program. It is recommended to use BioRad's CFX96, or QuantStudio's 7 Flex systems.

Steps	Temperature	Time	Cycles
cDNA synthesis	42°C	15 min	1
Pre-Denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing	60°C	60 sec	

Detection Channels:

- Four channels (FAM, HEX, ROX, & Cy5) are used in this one tube qPCR assay. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. **Please refer to the instrument's user manual to perform this calibration.**
- Choose the FAM, HEX, ROX, and Cy5 channels for each sample to be tested with this RT-qPCR Rapid Detection Kit.
- Select "None" for ROX passive reference on any Applied Biosystem's qPCR machine.

IX. 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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