FOR RESEARCH USE ONLY!

Coronavirus Rapid RT-qPCR Detection Kit

Rev *04/20*

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

I. Introduction:

BioVision's Coronavirus Rapid RT-qPCR Detection Kit is used for the detection of SARS-CoV-2 nucleic acid in human nasopharyngeal and oropharyngeal swab 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 a vial of primers and probes. The kit is highly specific for the RNA-dependent RNA polymerase (RdRP) and nucleocapsid (N) targets recommended by WHO and US CDC. During the amplification process, the included probes will anneal to the specific target sequence located between the forward and reverse primers. The probe is then cleaved, releasing the reporter dye and generating a fluorescent signal. An internal control primer and probe set (RP) is included to monitor proper specimen collection and assay setup. The PCR Detection Kit will facilitate research on Coronavirus research.

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 RdRP 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 nasopharyngeal and oropharyngeal swab samples. Flocked swabs are preferred. Sterile dacron or rayon swabs with plastic or flexible metal handles may also be used. Do NOT use cotton or calcium alginate swabs or swabs with wooden sticks as they may contain substances that inactivate viruses and inhibit PCR.

V. Kit Contents:

Components	K1461-100 (100 Rxns)	Part Number
COVID-19 Primers/Probes	200 µl	K1461-100-1
2X RT-qPCR Master Mix	1.25 ml	K1461-100--2
Positive Control Template	100 µl	K1461-100-3
Negative Extraction Control	1.0 ml	K1461-100-4
RT-qPCR Enzyme Mix	40 µl	K1461-100-5
Nuclease-free Water	1 ml	K1461-100-6

VI. User Supplied Reagents and Equipment:

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

VII. Shipping and Storage Conditions:

The kit should be stored at -20°C upon arrival. Avoid repeated freeze-thaw cycles. Keep the reagents on ice when thawed. Avoid prolonged exposure to light.

VIII. Assay Protocol:

1. **Sample Preparation:** Precautions must be taken to prevent cross-contamination of samples. To monitor that there is no cross - contamination during the extraction process, extract the Negative Extraction Control (K1461-100-4) included in this kit alongside your samples for each sample preparation run. Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hr, or at -70°C for long term storage. Separate work areas should be used for nucleic acid extraction and reagent preparation.

Notes:

a. Proper microbiological, aseptic technique should always be followed when working with RNA. Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from laboratory equipment.

b. During the procedure, work quickly and keep all reagents on cold blocks when possible to avoid degradation of RNA. Once the reagents have been thawed, vortex and centrifuge the tubes briefly before use.

c. Each process in the experiment should be conducted in different designated zones (reagent preparation zone, sample processing zone, amplification zone and product analysis zone). Always use sterile pipette tips with filters.

2. **RT-qPCR MasterMix Preparation:** Prepare sufficient quantity of the following reagent mix for the number of samples and controls being tested:

Reagent	Volume per reaction
COVID-19 Primers/Probes	2 µl
2X RT-qPCR MasterMix	10 µl
RT-qPCR Enzyme Mix	0.4 µl
Nuclease-free Water	2.6 µl

3. Add 15 µl of the RT-qPCR MasterMix prepared in Step 3 to required wells of PCR plate.
4. Add 5 µl of nuclease-free Water to the Negative Control well and cap accordingly. This is the no-template-control (NTC) reaction.

5. Move the PCR plate to Template Addition Room.
6. Add 5 µl of extracted nucleic acid from each sample to the test wells.
7. Add 5 µl of extracted nucleic acid from Negative Extraction Control to the Negative Extraction Control well.
8. Add 5 µl of Positive Control Template to the Positive Control well.
9. Cap all wells securely with optical caps or seal the plate with an optical film.
10. Centrifuge the PCR plate to collect all liquid in the bottom of the wells using a tabletop refrigerated centrifuge.
11. Transfer the PCR plate to a qPCR instrument.

IX. Standard RT-qPCR Cycling Condition:

Transfer the reaction setup into the 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: Three channels (FAM, HEX and ROX) are used in this single tube qPCR assay. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. Select "None" for ROX passive reference on any qPCR machines requiring ROX as reference dye.

X. Expected Performance of Controls:

Control Type	Used to Monitor	Expected Results and C_t Values		
		N (FAM)	RdRP (ROX)	RP (HEX)
Negative ("NTC")	Assay or extraction reagent contamination	Negative C_t ND	Negative C_t ND	Negative C_t ND
Positive	Improper assay setup and reagent failure, including primer and probe degradation	Positive C_t < 40.0	Positive C_t < 40.0	Negative C_t ND
Negative Extraction Control	Cross-contamination during extraction	Negative C_t ND	Negative C_t ND	Positive C_t < 40.0
Positive Extraction Control ("RP")	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, or PCR inhibition	Negative C_t ND	Negative C_t ND	Positive C_t < 40.0

ND= Not Detected. If any control does not perform as specified above, results are considered invalid.

XI. Interpretation of Results:

N	RdRP	RP	Interpretation
+	+	+/-	Positive
If only one of the two targets are positive		+/-	Inconclusive Result
-	-	+	Negative
-	-	-	Invalid Result

Limitation of Test Methods:

Possible causes for false negative results:

- Improper sample collection, transportation and treatment, and/or excessively low virus droplets in samples.
- Mutations in the target sequence of SARS-CoV-2 or changes in the sequence caused by other reasons.
- Other untested interferences or PCR inhibitors.

False positive results may occur if cross-contamination is not well managed during sample processing.

XII. 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

FOR RESEARCH USE ONLY! Not to be used on humans.