SARS-Cov-2 Coronavirus (Covid-19) Real-Time RT-PCR (RT-qPCR) Detection Kit

REF : KBRT010

Ver 4.0

IVD

SARS-CoV-2 Coronavirus (Covid-19) Real-Time RT-PCR (RT-qPCR) Detection Kit is designed to detect the presence of SARS-CoV-2 Coronavirus in respiratory specimens and serum sample as recommended by CDC (Centre for Disease Control) Atlanta, USA.

IVD	**For In-Vitro Diagnostic Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

For In-Vitro Diagnostic Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

CE

REF KBRT010 Σ 100 reactions

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Introduction:

Coronaviruses are a family of large RNA viruses with size ranging from 26 to 32 kb. These viruses are zoonotic and in human can cause respiratory infections. As the coronavirus is an RNA virus it has a relatively high mutation rate resulting in rapid evolution. In December 2019, a new deadly coronavirus known as SARS-CoV-2 (previously known as 2019-nCoV), which has a high sequence similarity to SARS-CoV, was identified and has caused a pneumonia, known as Covid-19, outbreak in Wuhan, China and spread globally.

Intended Use:

SARS-CoV-2 Coronavirus (Covid-19) Real-Time RT-PCR (RT-qPCR) Detection Kit is designed to detect the presence of SARS-CoV-2 Coronavirus in respiratory specimens and serum sample. The kit is designed as per guidelines issued by Centre for Disease Control (CDC), Atlanta, USA.

Principle:

This kit contain three primer/probe sets (N gene primer/probe set 1, and N gene primer/probe set 2 that target the conservative regions of coronavirus nucleocapsid (N) gene, the Human RPP30 gene primer/probe set (Human RPP30 gene primer/probe set) that targets exon 1 of human RPP30 gene and serves as a control to assess specimen quality and a non-infectious DNA positive control to ensure reagents and instruments are working properly. Please refer to Tables 4 and 5 for results interpretation.

Reagents and Materials Provided:

Sr No	Component	Quantity	Storage
N1	N gene Primer/Probe set 1, lyophilized	1 vial	-20°C
N2	N gene Primer/Probe set 2, lyophilized	1 vial	-20°C
RP	Human RPP30 gene Primer/Probe set, lyophilized	1 vial	-20°C
H2O	Nuclease-free H_2O	8 ml	4°C
Pos	Positive Control (non-infectious DNA)	200 ul	-80°C
MM	RT-qPCR Master Mix, 4X	500 ul	-20°C

Materials to be Provided by the End-User:

- QIAamp® DSP Viral RNA Mini Kit (Qiagen; catalog #61904)
- LightCycler® 96 Real-Time PCR System with LightCycler® Software 1.01.01.0050 (Roche; catalog #05815916001)
- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 ul, 200 ul and 1000 ul)
- Multichannel micropipettes (5-50 ul)
- Racks for 1.5 ml microcentrifuge tubes
- Aerosol barrier pipette tips
- 1.5 ml microcentrifuge tubes (DNase/RNase free)
- Disposable powder-free gloves and surgical gowns
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNAZap™ (Ambion; catalog #AM9890) or equivalent
- RNAse Away™ (Fisher Scientific; catalog #21-236-21) or equivalent.

Handling / Storage:

Store the Primer/Probes sets, N gene Primer/Probe set 1, N gene Primer/Probe set 2 at -20°C in a manual defrost freezer, and the Positive control at -80°C, and nuclease-free H_2O at 4°C.

Specimen Collection, Handling, and Storage:

- Human nasal, nasopharyngeal, oropharyngeal swab specimens, and bronchoalveolar lavage may be used with the Krishgen SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit.
- Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.
- Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.
- Specimens can be stored at 2-8 °C for up to 72 hours after collection. If a delay in extraction is expected, store specimens at -80 °C.
- Extracted nucleic acid should be stored at -80 °C.

Reagent Preparation:

No Template Control (NTC) Preparation

1. NTC is nuclease-free H2O (#H2O).
2. Aliquot in small volumes (approximately 900 ul each).

Primer/Probe Set Preparation

1. Upon receipt, store lyophilized primer/probe sets at -20 °C.
2. Prior to use, allow the primer/probe sets (#N1, #N2, and #RP) to warm to room temperature in the dark.
3. Centrifuge the vials at 1,500x g for 1 minute.
4. Add 200 ul nuclease-free H2O (#H2O) to **N gene Primer/Probe set 1 (lyophilized)** and allow to rehydrate for 15 min at room temperature to make N1 primer/probe stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Maintain cold and in the dark when thawed.
5. Add 200 ul nuclease-free H2O (#H2O) to **N gene Primer/Probe set 2 (lyophilized)** and allow to rehydrate for 15 min at room temperature to make N2 primer/probe stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Maintain cold and in the dark when thawed.
6. Add 200 ul nuclease-free H2O (#H2O) to **Human RPP30 gene Primer/Probe set (lyophilized)** and allow to rehydrate for 15 min at room temperature to make RP primer/probe stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Maintain cold and in the dark when thawed.

Positive Control Preparation

1. **Positive Control** is non-infectious RNA spiked into human small airway epithelial cells.
2. Aliquot in small volumes (approximately 18 ul each), and store at -80°C. Do not freeze-and-thaw for more than once.

Assay Procedure:

1. Prior to use, allow the **Primer/Probe sets (N1, N2 and RP)** to warm to room temperature in the dark.
2. Centrifuge the vials at 1,500 g for 1 minute.
3. Two control samples should be run concurrently, the **Positive Control**, and **H_2O** as the No Template Control (NTC). Prepare three RT-qPCR reactions for each control sample, one with **N1 Primer/Probe stock solution**, one with **N2 Primer/Probe stock solution**, and one with **RP Primer/Probe stock solution**. Prepare **20 ul RT-qPCR reactions** for one well as shown below.

Table 1.

Control Sample	5 ul
Primer/probe stock solution (N1, N2, N3 or	2 ul
1-step RT-qPCR Master mix, 4X	5 ul
Nuclease-free H_2O	8 ul
Total Volume	***20 ul***

4. For each extracted RNA test sample, prepare three RT-qPCR reactions, one with **N1 Primer/Probe stock solution**, one with **N2 Primer/Probe stock solution** and one with **RP Primer/Probe stock solution**. Prepare **20 ul RT-qPCR reactions** for one well as shown in below:

Table 2.

RNA test sample (concentration varies)	5 ul
Primer/Probe stock solution (N1, N2, N3 or RP)	2 ul
1-step RT-qPCR Master mix, 4x	5 ul
Nuclease-free H_2O	8 ul
Total Volume	***20 ul***

5. Seal the RT-qPCR reaction wells. Centrifuge the plates or tubes at 1,500 g for 15 seconds.
6. Setup RT-qPCR reactions as shown in Table 3.

Table 3.

Instrument Settings for RT-qPCR reactions. Fluorescence data (FAM) should be collected during the data acquisition step.

Step	Temperature	Time	Number of cycles
UNG Incubation	25°C	2 min	1
Reverse Transcription	50°C	15 min	1
Enzyme Activation	95°C	2 min	1
Denaturation	95°C	3 sec	45
Annealing and Extension	55°C	30 sec	
Data Acquisition	Plate read, detector (FAM)		

Typical Plate Schematic:

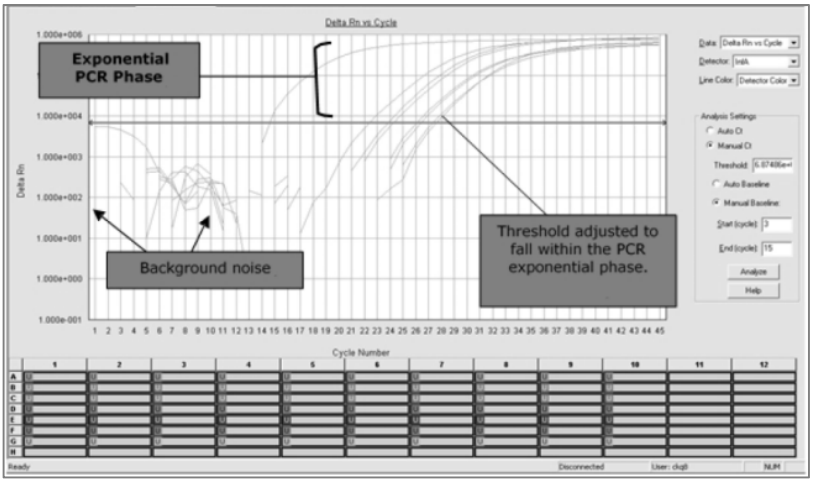
	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	S8	S8	S8	S16	S16	S16	S24	S24	S24
B	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
C	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26
D	S3	S3	S3	S11	S11	S11	S19	S19	S19	S27	S27	S27
E	S4	S4	S4	S12	S12	S12	S20	S20	S20	S28	S28	S28
F	S5	S5	S5	S13	S13	S13	S21	S21	S21	S29	S29	S29
G	S6	S6	S6	S14	S14	S14	S22	S22	S22	S30	S30	S30
H	S7	S7	S7	S15	S15	S15	S23	S23	S23	PC	PC	PC

S1	S2	S3
↑	↑	↑
S+N1	S+N2	S+RP

S= Test Sample
N1= N1 Primer/Probe Set
N2= N2 Primer/Probe Set
RP=RP Primer/Probe Set

Data Analysis:

After completion of the PCR run, save and analyze the data following the instrument manufacturer's instructions. Analysis should be performed separately for each target using a manual threshold setting. Thresholds (Ct Values) should be adjusted to fall within the exponential phase of the fluorescence curves and above any background signal (refer to image below). The procedure chosen for setting the threshold should be used consistently.



Interpretation of Results:

Table 4. Control Sample Results Interpretation.

Sample	KT909-N1	KT909-N2	KT-RP	Results Interpretation
2020-Pos	+	+	+	**Expected**
	-	-	-	**Reverse Transcription and/or PCR Failed**
NTC (H2O)	-	-	-	**Expected**
	If anyone of three targets is positive			**Reagent(s) Contaminated**

Note: a Ct value < 40.00 is considered positive.
Note: Any controls not showing the expected results is an indication that reagent(s) and/or equipment(s) are not functioning properly. The run is invalid and should be repeated.

Table 5. Patient Sample Results Interpretation when control results are as expected.

KT909-N1	KT909-N2	KT909-RP	Results Interpretation
+	+	±	**SARS-CoV-2 Detected**
If only one of two targets is positive		±	**SARS-CoV-2 Detected**
-	-	+	**SARS-CoV-2 Not Detected**
-	-	-	**Invalid Result. Repeat extraction and RT-qPCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.**

Note: a Ct value < 40.00 is considered positive.

Quality Control:

1. Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.
2. One positive control and one negative control (provided) must be processed with each run. Quality control procedures are intended to monitor reagent and assay performance. For control results interpretation, refer to section "Interpretation of Results".
3. Test positive control prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
4. Good laboratory practice (cGLP) recommends including the controls in each run.

Performance Characteristics

Limit of Detection (LoD):
LoD studies determine the lowest detectable concentration of SARS-CoV-2 that could be reliably detected at least 95% of the time. SARS-CoV-2 extracted RNA obtained with known titer (RNA copies/ul) was spiked into pooled nasopharyngeal matrix. Samples were extracted using the QIAGEN DSP Viral RNA Mini Kit (Qiagen; catalog #61904). RT-qPCR assays were performed on the Roche LightCycler® 96 Real-Time PCR System with LightCycler® Software 1.01.01.0050.

A preliminary LoD was determined testing four replicates of 3-fold serial dilutions of quantified SARS-CoV-2 extracted RNA. A confirmation of the LoD was determined using 3-fold serial dilution RNA samples with 20 extracted replicates. The LoD was determined as the lowest concentration where ≥ 95% (19/20) of the replicates were positive.

Limit of Detection confirmation of the SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit

Targets	KT909-N1				KT909-N2			
RNA Concentration (copies/ul)	10^1	100^0.5	10	10^-0.5	10^1.5	10^1	10^0.5	10^0
Positive/Total	20/20	20/20	19/20	43862	20/20	20/20	19/20	0/20
Mean Cta	27.68	28.76	30.50	NA	28.63	29.62	31.79	NA
Standard Deviation (Ct)	0.07	0.27	0.26	NA	0.21	0.14	1.02	NA

Limit of Detection of the SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit with viral RNA is **$10^{0.5}$ copies / ul**.

Cross-Reactivity:
The SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit utilizes oligos that have identical sequences to those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel the CDC assay.

Endogenous Interference Substances Studies:
The SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit uses conventional well-established nucleic acid extraction method that is also authorized with the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel in the CDC assay. We do not anticipate interference from common endogenous substances using this method.

Clinical Performance:
Clinical evaluation of the SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit was conducted using contrived nasopharyngeal swabs (30 positives and 30 negatives). The 30 positive samples were spiked by SARS-CoV-2 extracted RNA obtained, 20 of the contrived positive samples (Samples #1-20) were prepared by spiking the SARS-CoV-2 RNA at 1x – 2x LoD and the rest (Samples #21 -30) 10 contrived positive samples spanned the testing range of the assay (< 5x LoD). The positive and negative agreements between the SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit and the expected results are shown below:

Summary:

SARS-CoV-2 Concentration	Results (Detected / Tested)	Agreements (95% CI)
1-2x LoD	20/20	100% (83.9%, 100%)
3x-5x LoD	10/10	100% (72.3%, 100%)
Negative	30/30*	100% (88.7%, 100%)

*Negative result detected / tested

Warnings and Precautions

- **The regulatory status defers in each country. The Kit is CE marked "For Diagnostic Use" Only.** ***Note in the US, it is For Research Use Only.*** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition https://www.cdc.gov/labs/BMBL.html.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Laboratories within each country and its territories are required to report all positive results to the appropriate public health authorities.
- PCR-based detection technologies are sensitive to accidental contamination of previous PCR products. False positive results could occur if either the clinical specimen or the real-time reagents become contaminated.
- Perform for assay setup and handling of nucleic acids in separate areas. Workflow in the laboratory should proceed in a unidirectional manner. Use separate and dedicated equipment and supplies in each area.
- Do not substitute or mix reagent from different kit lots or from other manufacturers. Only use aerosol barrier pipette tips and change tips between liquid transfers.
- Good laboratory techniques should be followed to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples. Proper aseptic technique should always be used when working with nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves when setting up assays, and change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.
- Dispose of unused kit reagents and human specimens according to local, state, and central regulations.

Limitations

- The use of SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit as an *in vitro* diagnostic is limited to laboratories that are certified under CLIA and permitted under each country's regulatory regime.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- A **false negative result** may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- A **false positive result** may arise from cross contamination during specimen handling or preparation, or between patient samples.

- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- Results from the SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit should be used as an adjunct to clinical observations and other information available to the physician.

References:

1. Ballew, H. C., et al. “Basic Laboratory Methods in Virology,” DHHS, Public Health Service 1975 (Revised 1981), Centers for Disease Control and Prevention, Atlanta, Georgia 30333.
2. Clinical Laboratory Standards Institute (CLSI), “Collection, Transport, Preparation and Storage of Specimens for Molecular Methods: Proposed Guideline,” MM13-A.
3. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. 2020, US Centers for Disease Control and Prevention. https://www.fda.gov/media/134922/download
4. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primer and Probe Information. 2020, US Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf

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