PowerChek™ 2019-nCoV Real-time PCR Kit

Instructions for use

for use under Emergency Use Authorization only

for *in vitro* diagnostic (IVD) use

for Prescription (Rx) use only

REF **R6900TD**

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1. Intended Use

PowerChek™ 2019-nCoV Real-time PCR Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior/mid-turbinate nasal swabs, nasopharyngeal/oropharyngeal swabs, nasopharyngeal washes/aspirates or nasal aspirates, bronchoalveolar lavage (BAL), and sputum from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detected in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the PowerChek™ 2019-nCoV Real-time PCR Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The PowerChek™ 2019-nCoV Real-time PCR Kit is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

2. Product description

PowerChek™ 2019-nCoV Real-time PCR Kit provides a testing solution for SARS-CoV-2, including the assays and controls for a multiplex real-time RT-PCR test for the qualitative detection of RNA from SARS-Cov-2 in the following upper and lower clinical specimens: anterior/mid-turbinate nasal swabs, nasopharyngeal/oropharyngeal swabs, nasopharyngeal washes/aspirates or nasal aspirates, bronchoalveolar lavage (BAL) fluid, and sputum obtained from individuals suspected of COVID-19 by their healthcare provider. The RdRP target is specific for SARS-CoV-2 whereas the E gene is specific for Sarbecovirus. Both assays include a heterologous amplification system (Internal Control) to identify possible inhibition of nucleic acid amplification and to confirm the integrity of the reagents of the kit.

The test is based on real-time RT-PCR technology, utilizing reverse transcription (RT) to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes. In both assays, probes specific for SARS-CoV-2 or Sarbecovirus RNA are labeled with the fluorophore **FAM**. The probe specific for the target of the Internal Control (IC) is labeled with the fluorophore **JOE**. Using probes linked to distinguishable dyes enables the parallel detection of SARS-CoV-2 specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

3. Contents and storage

The PowerChek™ 2019-nCoV Real-time PCR Kit can be shipped and stored at –25 to –15℃ until the expiration date printed on the label. All components of the PowerChek™ 2019-nCoV Real-time PCR Kit must be stored at –25 to –15℃, before and after opening (Table 1). Do not repeat the freeze/thaw procedure more than 3 times after opening. Exposure to light, heat, or humidity may affect the shelf life of some of the kit components and should be avoided.

Table 1. Components of PowerChek™ 2019-nCoV Real-time PCR Kit (R6900TD) for 100 reactions

Reagent	Cap label	Volume	Description	Storage
Primer/Probe Mix 1 (E gene)	P1	400 µL	- E specific primers and probe - IC specific primers and probe - DNA for IC	–25 to –15℃
Primer/Probe Mix 2 (RdRP gene)	P2	400 µL	- RdRP specific primers and probe - IC specific primers and probe - DNA for IC	–25 to –15℃
RT-PCR Premix	RP	2 X 1100 µL	Buffer containing dNTPs, $MgCl_2$, *Taq*. One-step RT Real-time PCR Enzyme Mix	–25 to –15℃
Control 1 (E gene)	C1	100 µL	Positive control RNA (E gene), (2×10^4 copies/ µL)	–25 to –15℃
Control 2 (RdRP gene)	C2	100 µL	Positive control RNA (RdRP gene) (2×10^4 copies/ µL)	–25 to –15℃

4. Additionally required materials (but not provided)

- **PowerChek™ RNA Process Control Kit (KogeneBiotech, Cat No. IC0002 for 500 reactions)**
  - The PowerChek™ RNA Process Control Kit must be used with the PowerChek™ 2019-nCoV Real-time PCR Kit (R6900TD) to monitor the RNA extraction process as well as RT-PCR inhibition.
  - The PowerChek™ RNA Process Control Kit consists of the RNA Process Control (RPC), a nuclease-resistant artificial RNA, and the Primer/Probe mix (RNA Process Control) specific to the RNA Process Control. The RPC has no homologies to any other known sequences or the Internal Control of the PowerChek™ 2019-nCoV Real-time PCR Kit (R6900TD).

- The Components of PowerChek™ RNA Process Control Kit (for 500 reactions)

Reagent	Volume
Primer/Probe mix (RNA Process Control)	250 µL
RNA Process Control (RPC)	5 X 500 µL

- The set of primers and probes used in the PowerChek™ RNA Process Control Kit has no effect on the amplification of SARS-CoV-2 RNA and the specific probe for the RPC is labeled with fluorophore Cy5 so that the co-amplification of SARS-CoV-2 RNA, IC, and RPC is detected through the **FAM, JOE (or HEX)**, and **Cy5** channels, respectively.

Unless otherwise indicated, all materials are available from major laboratory suppliers.

- Real-time PCR instrument and equipment
  - Applied Biosystems™ 7500 Real-time PCR System
  - Applied Biosystems™ 7500 fast System run as a standard AB 7500
  - CFX96™ Real-time PCR Detection System
- Laboratory freezers
  - –30°C to –10°C
  - ≤ –70°C
- Centrifuge with a rotor for 2 mL reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Laboratory mixer, Vortex or equivalent
- Pipettes (adjustable 1.00 µL to 1,000.00 µL)
- Cold block or ice
- Appropriate nucleic acid extraction system or kit:
  - QIAamp® DSP Viral RNA Mini Kit (QIAGEN, Cat. No./ID: 60704)
- Nuclease-free water (not DEPC-treated)
- Disposable polypropylene micro-tubes or 96 well reaction plates with corresponding (optical) closing materials
  - For Applied Biosystems™ 7500 Real-time PCR System:
    - MicroAmp™ Optical 96-Well Plate (Applied Biosystems™, Cat. No. 8010560, 4316813)
    - MicroAmp™ Optical 96-Well Plate with Barcode (Applied Biosystems™, Cat. No. 4306737, 4326659)
    - MicroAmp™ Optical Adhesive Film (Applied Biosystems™, Cat. No. 4311971)

- MicroAmp™ Optical 8-Tube Strip, 0.2-mL (Applied Biosystems™, Cat. No. 4316567)
- MicroAmp™ Optical 8-Cap Strip (Applied Biosystems™, Cat. No. 4323032)

○ For Applied Biosystems™ 7500 Fast Real-time PCR System:

- MicroAmp™ Fast Optical 96-Well Plate, 0.1 mL (Applied Biosystems™, Cat. No. 4346907)
- MicroAmp™ Fast Optical 96-Well Plate with Barcode, 0.1 mL (Applied Biosystems™, Cat. No. 4346906, 4366932)
- MicroAmp™ Optical Adhesive Film (Applied Biosystems™, Cat. No. 4311971)
- MicroAmp™ Fast 8-Tube Strip, 0.1 mL (Applied Biosystems™, Cat. No. 4358293)
- MicroAmp™ Optical 8-Cap Strip (Applied Biosystems™, Cat. No. 4323032)

○ For CFX96™ Real-time PCR Detection System:

- Low-Profile 8-Tube Strips without Caps, white (Bio-Rad, Cat. No. TLS0851)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Bio-Rad, Cat. No. HSP9655)
- Optical Flat 8-Cap Strips (Bio-Rad, Cat. No. TCS0803)

- Sterilize aerosol barrier (filtered) pipette tips
- Disposable powder-free gloves and laboratory coat

5. Warning and Precautions

- For in vitro diagnostic use (IVD) only.
- For Emergency Use Authorization only.
- For prescription use only.
- This product has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

- Specimens should always be considered potentially infectious and handled in accordance with safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with 2019-nCoV is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents and human specimens are handled.
- Perform all manipulations of live virus samples within a class II (or higher) biological safety cabinet.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Wash hands thoroughly after handling specimens and reagents.
- The PowerChek™ 2019-nCoV Real-time PCR Kit is a single-use device.
- All samples and positive/negative controls should be tested with **both** RdRP and E gene amplification mixtures in order to generate valid results. The two amplification reactions should not be used independently.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Do not use a kit after its expiration date. The product will maintain performance through the control date printed on the label.
- Material Safety Data Sheets (MSDS) can be requested through KogeneBiotech website (www.kogene.co.kr/eng) or e-mail (info@kogene.co.kr).
- The laboratory process must be one-directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment, and reagents in the area where you performed the previous step.
- Please be careful not to contaminate the Primer/Probe Mixture and RT-PCR premix with PCR products or Control DNA through pipetting. To prevent contamination, use of filter tips is recommended.
- Store extracted positive materials (samples, controls, and other amplicons) away from all other reagents and add it to the reaction mix in a separate area.

- Thaw all components thoroughly on ice before starting, experiment. When thawed, mix the components and centrifuge briefly.
- Dispose of all samples that have come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectants.
- Avoid contact of specimens and reagents with the skin, eyes, and mucosa. If contact with skin, eyes, and mucosa, immediately flush with water and seek medical attention.

6. Sample collection, transport, and storage

The sample collection, storage, and handling must be performed according to the CDC guidelines (Table 2). If samples cannot be processed immediately upon receipt or if there is a delay in shipping, specimens should be stored at 4℃ if the delay is expected to last less than 72 hours. If the delay in processing is expected to exceed 72 hours, specimens should be stored at -70℃ until processing or shipping can commence. For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements and avoid repeated freezing and thawing of specimens.

Table 2. Recommended specimen collection, transport, and storage conditions

Specimen type	Collection materials	Transport to laboratory	Storage till testing
Nasopharyngeal and oropharyngeal swabs	Dacron or polyester flocked swabs	4℃ (or iced)	≤ 72 hours at 4℃ > 72 hours at –70℃
Bronchoalveolar lavage	Sterile container	4℃ (or iced)	≤ 72 hours at 4℃ > 72 hours at –70℃
Sputum	Sterile container	4℃ (or iced)	≤ 72 hours at 4℃ > 72 hours at –70℃

NOTE: Sample collection devices are not provided with the PowerChek™ 2019-nCoV Real-time PCR Kit. All testing for COVID-19 should be conducted in consultation with a healthcare provider. Refer to CDC guidelines for sample collection and storage at: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

7. Samples and Controls

Patient samples must be collected according to appropriate laboratory guidelines. Positive and negative test controls must be included to accurately interpret patient test results. **An internal amplification control (IC)** is included in the Primer/Probe Mix 1 (E gene) and the Primer/Probe Mix 2 (RdRP gene) and used to monitor for the presence of PCR inhibitors in specimens. The IC can be co-amplified with target DNA at the same time and visualized in the JOE (or HEX) channel.

Included the following controls when testing clinical samples:

1) **Positive Controls:** Two positive controls, Control 1 (E gene) and Control 2 (RdRP gene) are needed to verify the functionality of the SARS-CoV-2 specific RT-PCR detection system and must be included in each test batch. The positive controls are synthesized RNA by *in vitro* transcription and contain the specific target region for the E gene or RdRP gene.

2) **Negative Control (NTC):** A negative control is needed to check for contamination during assay preparation. The negative control must be included in each test batch. Molecular-grade water such as nuclease-free water should be used instead of nucleic acid template ("no template").

3) **Extraction control (RNA Process Control):** The RNA Process Control in the PowerChek™ RNA Process Control Kit (Kogenebiotech, Cat. No. IC0002) contains a defined copy number of a nuclease-resistant RNA and must be added to the clinical sample prior to RNA extraction. The RPC-specific probe is labeled with a different fluorophore, Cy5, which allows detection to be distinguished from the SARS-CoV-2/Sarbecovirus (FAM) and IC (JOE).

4) **Negative process control (NPC):** The RPC-spiked nuclease-free water serves as a negative extraction control to monitor for any cross-contamination during the extraction process. It also serves as an extraction control to validate extraction reagents and successful RNA extraction. The negative process control must be included in each test batch.

8. Workflow

Extracted RNA is the starting material for the PowerChek™ 2019-nCoV Real-time PCR Kit. The quality of the extracted RNA has a profound impact on the performance of the entire test system. The QIAamp® DSP Viral RNA Mini Kit (QIAGEN) has been validated for use with the PowerChek™ 2019-nCoV Real-time PCR Kit.

The PowerChek™ RNA Process Control Kit (KogeneBiotech, IC0002) must be used with the PowerChek™ 2019-nCoV Real-time PCR Kit (R6900TD) to monitor the RNA extraction process as well as RT-PCR inhibition. Add the RNA Process Control (RPC) to each clinical sample prior to the extraction process.

The RPC is co-amplified with SARS-CoV-2 RNA and the IC and is visualized in the Cy5 channel. Please refer to Section 4 (Additionally required materials (but not provided)). If you have any questions on the RNA Process Control, please contact our Technical Support (see Section 17).

Add the RNA Process Control (RPC) to a patient sample

(5 µL of RPC for each sample)

▼

Extract RNA from a patient sample using the QIAamp® DSP Viral RNA Mini Kit

▼

Prepare master mix (15.5 µL) and add extracted RNA (4.5 µL) to make 20 µL total reaction volumes

▼

Perform RT-PCR using the appropriate real-time instrument

▼

Analyze data and interpret the results of the patient's sample

9. Nucleic acid extraction

Nucleic acids are isolated and purified from anterior/mid-turbinate nasal swabs, nasopharyngeal or oropharyngeal swabs, nasopharyngeal washes/aspirates or nasal aspirates, as well as sputum and bronchoalveolar lavage fluid specimens using the QIAamp® DSP Viral RNA Mini Kit.

Prior to the start of the nucleic acid extraction, 5 µL of RNA Process Control (RPC) is directly added to the **dinical sample (140 µL of sample)**. The 5 µL of RNA Process Control (RPC) should be added to the nuclease-free water (140 µL) to serve as the Negative Process Control. The RPC-spiked nuclease-free water should be included in the extraction process as the Negative Process Control to monitor for any cross-contamination that may occur during the extraction process. It also functions as an extraction control to assess extraction reagent integrity and successful RNA extraction.

Utilize 140 µL of clinical sample and **elute with 60 µL of Buffer AVE** from the QIAamp® DSP Viral RNA Mini Kit. If the extracted RNA cannot be used immediately, store at 2 to 8℃ for up to 24 hours or at -70℃ for up to 1 month.

Instructions for extracting RNA using the QIAamp® DSP Viral RNA Mini Kit can be obtained from the QIAamp DSP Virus Kit Handbook.

10. Procedure

We recommend that all experiment steps be performed with poly-gloves to prevent the risk of contamination with nucleases (RNase or DNase).

***Caution:** Both reaction mixtures must be used for all samples and Positive/Negative Controls.

(1) Thaw the kit components on ice. Using ice is recommended during the experiment to maintain the enzyme activity. When thawed, mix the components and centrifuge briefly.

(2) Preparing PCR Mixture: Prepare the reaction mixtures for a 20 µL total reaction volume as shown in Table 3 below.

Table 3. Reaction mixture set-up for the PowerChek™ 2019-nCoV Real-time PCR Kit if RPC is added to samples to monitor the successful recovery of RNA as well as RT-PCR inhibition

Composition	Volume per reaction for E gene assay	Volume per reaction for RdRP gene assay
Primer/Probe Mix 1 (E gene) or Primer/Probe Mix 2 (RdRP gene)	4 µL	4 µL
Primer/Probe Mix (RNA Process Control)	0.5 µL	0.5 µL
RT-PCR Premix	11 µL	11 µL
Total volume	15.5 µL	15.5 µL

(2-1) If the RNA Process Control (RPC) is used to monitor ONLY the inhibition of reverse transcription, prepare two master mixes; one with 0.5 µL of RPC per reaction and one master mix without RPC addition. The one with no RPC is for the negative process control and follows the set-up in Table 3 and the other master mix with the addition of RPC is for the clinical samples and follows the set-up in Table 4.

Table 4. The reaction mixtures of PowerChek™ 2019-nCoV Real-time PCR Kit, if RPC is added to the PCR mixture to monitor the inhibition of reverse transcription

Composition	Volume per reaction for E gene assay	Volume per reaction for RdRP gene assay
Primer/Probe Mix 1 (E gene) or Primer/Probe Mix 2 (RdRP gene)	4 µL	4 µL
Primer/Probe Mix (RNA Process Control)	0.5 µL	0.5 µL
RNA Process Control	0.5 µL	0.5 µL
RT-PCR Premix	11 µL	11 µL
Total volume	16 µL	16 µL

(3) Prior to moving to the nucleic acid handling area, prepare the No Template Control (NTC) and Negative Process Control (NPC) by adding 4.5 µL of nuclease-free water and extracted RNA from the RPC-spiked nuclease-free water to the negative sample wells. Securely cap the NTC and NPC wells before processing.

(4) Add 4.5 µL of extracted RNA to each well containing the reaction mixture. An example of a reaction plate set-up per batch test is displayed in Figure 1.

96 well Plate	E gene assay (Primer/Probe Mix 1)						RdRp gene assay (Primer/Probe Mix 2)					
	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	*S8*	*S16*	*S24*	*S32*	*S40*	PC	*S8*	*S16*	*S24*	*S32*	*S40*
B	*S1*	*S9*	*S17*	*S25*	*S33*	*S41*	*S1*	*S9*	*S17*	*S25*	*S33*	*S41*
C	*S2*	*S10*	*S18*	*S26*	*S34*	*S42*	*S2*	*S10*	*S18*	*S26*	*S34*	*S42*
D	*S3*	*S11*	*S19*	*S27*	*S35*	*S43*	*S3*	*S11*	*S19*	*S27*	*S35*	*S43*
E	*S4*	*S12*	*S20*	*S28*	*S36*	*S44*	*S4*	*S12*	*S20*	*S28*	*S36*	*S44*
F	*S5*	*S13*	*S21*	*S29*	*S37*	*S45*	*S5*	*S13*	*S21*	*S29*	*S37*	*S45*
G	*S6*	*S14*	*S22*	*S30*	*S38*	NPC	*S6*	*S14*	*S22*	*S30*	*S38*	NPC
H	*S7*	*S15*	*S23*	*S31*	*S39*	NC	*S7*	*S15*	*S23*	*S31*	*S39*	NC

Figure 1. Example of reaction plate set-up

(5) Prepare the 1,000-fold diluted positive control samples by 10-fold serial dilution (<5X LoD).

(6) Next, add the 4.5 µL of each diluted positive control sample, Control 1 (for E gene) or Control 2 (for RdRP gene) to the wells for the positive control samples.

(7) After closing the PCR reaction tubes or 96 well reaction plate, mix the reagents in the PCR reaction tubes by tapping the tubes. Centrifuge for 5 seconds to remove air bubbles and to collect the contents at the bottom of the tubes/wells.

(8) Running the Real-time PCR instrument: Set up the PCR protocols according to Table 5. The PowerChek™ 2019-nCoV Real-time PCR Kit has been validated for use on the Applied Biosystems 7500 Real-time PCR System, the Applied Biosystems 7500 Fast Real-time PCR System, and the CFX-96 Real-time PCR Detection System. Read the manufacturer's instrument manual before running the test.

Table 5. Real-time PCR protocol for the PowerChek™ 2019-nCoV Real-time PCR kit Using the 3 Validated Instruments

Temperature	Time	Cycle
50 ℃	30 min	1
95 ℃	10 min	1
95 ℃	15 sec	40
60 ℃ **	1 min	

** Detect the fluorescence at this step.

(9) The fluorescence curves are analyzed in the FAM, JOE (or HEX), and Cy5 fluorescence detection channels as follows in Table 6.

Table 6. Specific Detection in Fluorescence Channels

E gene assay (Primer/Probe Mix 1)		**RdRP gene assay (Primer/Probe Mix 2)**	
Target Gene	**Fluorophore**	**Target Gene**	**Fluorophore**
E gene	FAM	RdRP gene	FAM
IC	JOE (or HEX)	IC	JOE (or HEX)
RNA Process Control	Cy5	RNA Process Control	Cy5

11. Data analysis

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

When the run finishes, set the threshold and baseline for each target gene as follows in Table 7.

Table 7. Analysis setting

Instrument	**Target**	**Threshold**
Applied Biosystems™ 7500 and 7500 Fast Real-time PCR System (Thermo Fisher Scientific)	E gene	0.2
	IC	0.2
	RNA Process Control	0.2
	RdRP gene	0.2
	IC	0.2
	RNA Process Control	0.2
CFX96™ Real-time PCR Detection System (Bio-Rad)	E gene	100
	IC	150
	RNA Process Control	200
	RdRP gene	100
	IC	150
	RNA Process Control	200

12. Quality Control

- Positive Control (PC): Two positive controls, Control 1 (E gene) and Control 2 (RdRP gene) are needed to verify the functionality of the SARS-CoV-2 specific RT-PCR detection system and must be included in each test batch. The positive controls are synthesized RNA by *in vitro* transcription and contain the specific target region for the E gene or RdRP gene.

- Negative Control (NC): PCR grade water is to be used as a negative control for the real-time PCR reaction. Its function is to monitor for reagent contamination.

- Negative Process Control (NPC): The RNA Process Control (RPC)-spiked nuclease-free water serves as a negative extraction control to monitor for potential cross-contamination during the extraction process. It also functions as an extraction control to verify extraction reagent integrity and successful RNA extraction. The extracted RPC is amplified and visualized in the Cy5 channel.

- Internal Control (IC): The Internal Control (IC) contains a defined copy number of an "artificial" DNA molecule that has no homologies to any other known sequences. The set of primers and probe and DNA molecules specific to the IC are included with an optimal concentration in Primer/Probe Mix 1 (E gene) and Primer/Probe Mix 2 (RdRP gene), so that the IC can be co-amplified with target DNA at the same time. The results can be visualized in the JOE (or HEX) channel. The IC is used to monitor for the presence of PCR inhibitors in specimens.

13. Results interpretation for patients

Assessment of clinical specimen test results must be performed after the positive and negative controls and the negative process control have been examined and determined to be valid and acceptable (Table 8). If the controls are not valid, the patient results cannot be interpreted.

Table 8. Validity of the diagnostic test run

	E gene Assay			RdRP gene Assay		
Control ID	**E gene (FAM)**	**RPC[2)] (Cy5)**	**IC (JOE)**	**RdRP gene (FAM)**	**RPC[2)] (Cy5)**	**IC (JOE)**
Positive Control (PC)	Ct value ≤ 37	Ct value > 35 or Not detected	Ct value ≤ 28	Ct value ≤ 37	Ct value > 35 or Not detected	Ct value ≤ 28
Negative Control (NC)[1)]	Ct value > 37 or Not detected	Ct value > 35 or Not detected	Ct value ≤ 28	Ct value > 37 or Not detected	Ct value > 35 or Not detected	Ct value ≤ 28
Negative Process Control (NPC)	Ct value > 37 or Not detected	Ct value ≤ 35	Ct value ≤ 28	Ct value > 37 or Not detected	Ct value ≤ 35	Ct value ≤ 28

[1)] If the NC exhibits a Ct ≤ 35 for E/RdRP/RPC, sample contamination may have occurred. Invalidate the run and repeat the assay using a different molecular biology grade water.

[2)] If the RNA Process Control (RPC) is added to the PCR mixture as following Table 4, the RPC should be positive (Ct ≤ 35) in both positive and negative controls.

In the case of an invalid diagnostic test run, re-test using the remaining extracted nucleic acids. If the RNA Process Control (RPC) is negative in the Negative Process Control, repeat the nucleic acid extraction from the residual patient sample and re-test again. If a test run is repeatedly invalid, please contact our Technical Support (see Section 17).

The result of each target in clinical samples can be determined by the criteria described in Table 9 below. The expected results for the PowerChek™ 2019-nCoV Real-time PCR Kit are shown in Table 10. If results are obtained that do not follow these guidelines, re-extract from the residual patient sample and re-test the sample. If the repeat result remains invalid, consider collecting a new patient specimen.

Table 9. The criteria of each target; E gene, RdRP, IC, and RPC

	E gene	RdRP gene	RPC	IC
Positive (+)	Ct value ≤ 37	Ct value ≤ 37	Ct value ≤ 35	Ct value ≤ 28
Negative (–)	Ct value > 37 or Not detected	Ct value > 37 or Not detected	Ct value > 35 or Not detected	Ct value > 28 or Not detected

Table 10. Results interpretation as regarding the Ct values above

Case	E gene			RdRP gene			Interpretation	Action
	E (FAM)	RPC (Cy5)	IC (JOE)	RdRP (FAM)	RPC (Cy5)	IC (JOE)		
1	-	+	+	-	+	+	"SARS-CoV-2 is NOT detected".	Report the result to sender.
2	+	+/-*	+/-*	+	+/-*	+/-*	"SARS-CoV-2 is detected".	Report the result to sender and appropriate public health authorities.
3	-	+	+/-*	+	+/-*	+/-*	"SARS-CoV-2 is detected".	Report the result to sender and appropriate public health authorities. Missing amplification of E gene target may be due to: 1) a low concentration of viral RNA, 2) a mutation in the corresponding target region, or 3) other factors.
4	+	+/-*	+/-*	-	+	+/-*	Presumptive positive to SARS-CoV-2	Repeat the extraction from the residual patient sample and test again. If the result is still "Presumptive positive", report result to sender and appropriate public health authorities. Missing amplification of RdRP gene target may be due to: 1) a low concentration of viral RNA, 2) a mutation in the corresponding target region, 3) possible infection of other Sarbecovirus, or 4) other factors.

5	-	-	+/-	-	-	+/-	Invalid result	Repeat extraction from the residual patient sample and test again. Poor RNA yield or RT-PCR inhibition is suspected. If the repeated result is still "invalid", report the result to the sender and give the recommendation of the collection of a new specimen.

* Detection of the Internal Control (IC) in the JOE detection channel and/or RNA Process Control (RPC) in the Cy5 channel is not required for positive SARS-CoV-2 results. A high copy number of target-specific gene can lead to reduced or absent IC or RPC.

14. Assay limitations

- The performance of the PowerChek™ 2019-nCoV Real-time PCR Kit was established using nasopharyngeal swabs and sputum samples only.
- Anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage specimens are considered acceptable specimen types for use with the PowerChek™ SARS-CoV-2 Real-time PCR Kit but performance has not been established.
- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross-react with the RdRP primer set of the PowerChek™ 2019-nCoV Real-time PCR Kit. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- 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. Refer to the CDC guidelines for sample collection and storage at: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The PowerChek™ 2019-nCoV Real-time PCR Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2, and should not be the sole

basis of a patient management decision.

- Laboratories are required to report all results to the appropriate public health authorities.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Specimen collection after nucleic acid can no longer be found in the specimen matrix
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross-contamination during specimen handling or preparation
  - Cross-contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling
- This assay must not be used on the specimen directly. The nucleic acid extraction kit that was validated for use with the PowerChek™ 2019-nCoV Real-time PCR Kit is the QIAamp® DSP Viral RNA Mini Kit.
- The E gene assay of PowerChek™ 2019-nCoV Real-time PCR Kit can detect the Sarbecovirus including SARS, SARS-CoV-2, and SARS-related coronavirus. The detected signal of the E gene target in the FAM channel could indicate the presence of SARS coronavirus or SARS-related coronavirus.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

15. Conditions of authorization for laboratories

The PowerChek™ 2019-nCoV Real-time PCR Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the PowerChek™ 2019-nCoV Real-time PCR Kit, the relevant Conditions of Authorization are listed below.

- Authorized laboratories[1] using the PowerChek™ 2019-nCoV Real-time PCR Kit will include with test result reports of the PowerChek™ 2019-nCoV Real-time PCR Kit, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the PowerChek™ 2019-nCoV Real-time PCR Kit will perform the PowerChek™ 2019-nCoV Real-time PCR Kit as outlined in the PowerChek™ 2019-nCoV Real-time PCR Kit Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to perform the PowerChek™ 2019-nCoV Real-time PCR Kit are not permitted.
- Authorized laboratories that receive the PowerChek™ 2019-nCoV Real-time PCR Kit will notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the PowerChek™ 2019-nCoV Real-time PCR Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and KogeneBiotech (info@kogene.co.kr) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

- KogeneBiotech, its authorized distributor(s) and authorized laboratories using the PowerChek™ 2019-nCoV Real-time PCR Kit will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1]For ease of reference, this refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests as "authorized laboratories."

16. Performance characteristics

- **Analytical Sensitivity**

LoD studies using clinical sputum

The LoD study established the lowest SARS-CoV-2 viral RNA concentration (copies/μL) that consistently yielded a 95% positivity rate with the PowerChek™ 2019-nCoV Real-time PCR Kit.

A preliminary LoD for the SARS-CoV-2 specific target (RdRP gene) and the Sarbecovirus/SARS-CoV-2 target (E gene) was determined using viral genomic SARS-CoV-2 RNA (Human coronavirus (BetaCoV/Korea/KCDC03/2020)) from the National Culture Collection for Pathogens (NCCP). Clinical sputum matrices were screened negative by the PowerChek Kit and spiked with viral genomic RNA at various concentrations (2-fold dilutions) and tested in triplicate. RNA was extracted using the QIAamp DSP Viral RNA Mini Kit and run on the Applied Biosystems™ 7500 Real-time PCR System, Applied Biosystems™ 7500 Fast Real-time PCR System, and CFX96™ Real-time PCR Detection System (Table 11). The preliminary sputum LoD was determined to be 4 copies/μL on all PCR platforms.

The LoD of the PowerChek 2019-nCoV Real-time PCR Kit was confirmed using 20 individual extraction replicates consisting of spiked sputum samples at the LoD concentration. Samples were extracted with the QIAamp DSP Viral RNA Mini Kit and tested on the claimed Applied Biosystems and Bio-Rad PCR instruments. The lowest target level at which more than 95% of 20 replicates for sputum specimens produced positive results was 4 copies/μL for both all PCR platforms (Tables 12-14).

Table 11. The preliminary LoD study of the PowerChek™ 2019-nCoV Real-time PCR Kit (Sputa)

Concentration (copies/ µL)		Applied Biosystems™ 7500 Real-time PCR System			Applied Biosystems™ 7500 Fast Real-time PCR System			CFX96™ Real-time PCR Detection System		
		N	Mean Ct**	SD	N	Mean Ct	SD	N	Mean Ct	SD
E gene	8	3/3	35.24	0.12	3/3	35.02	0.24	3/3	35.51	0.02
	4	3/3	36.07	0.51	3/3	35.95	0.36	3/3	36.22	0.28
	2	1/3	NA*	NA	1/3	NA	NA	1/3	NA	NA
RdRP gene	8	3/3	35.21	0.37	3/3	35.32	0.11	3/3	35.64	0.34
	4	3/3	36.43	0.16	3/3	36.73	0.09	3/3	37.16	0.54
	2	1/3	NA	NA	1/3	NA	NA	1/3	NA	NA

* NA: not applicable

** Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

Table 12. LoD results on Applied Biosystems™ 7500 Real-time PCR System

Concentration	Target	Mean Ct (n=20)			% Positive
		E or RdRP	RPC	IC	
4 copies/µL	E gene	35.90	28.25	20.31	100
	RdRP gene	36.79	28.35	20.19	100

Table 13. LoD results on Applied Biosystems™ 7500 Fast Real-time PCR System

Concentration	Target	Mean Ct (n=20)			% Positive
		E or RdRP	RPC	IC	
4 copies/µL	E gene	35.92	28.65	20.52	100
	RdRP gene	36.53	28.22	20.22	100

Table 14. LoD results on CFX96™ Real-time PCR Detection System

Concentration	Target	Mean Ct (n=20)			% Positive
		E or RdRP	RPC	IC	
4 copies/µL	E gene	36.08	28.57	21.13	100
	RdRP gene	37.17	28.26	20.89	100

- **Inclusivity**

To demonstrate the predicted inclusivity of the PowerChek™ 2019-nCoV Real-time PCR Kit, *in silico* analysis was performed to verify primer and probe sequence homology with 10,252 whole-genome sequences of the SARS-CoV-2 in GISAID (n = 10007) and NCBI (n = 245) databases as of June 01, 2020. As a result, >99.6% of sequences exhibited 100% homology with the RdRP and E gene primers and probes (Table 15).

Table 15. *In silico* analysis for detection of SARS-CoV-2 sequences

Target	Primer or probe	Number of sequences	% of sequences exhibited 100% homology
E gene	Forward	10252 sequences	99.93%
	Reverse	10252 sequences	99.99%
	Probe	10252 sequences	99.95%
RdRP gene	Forward	10252 sequences	99.76%
	Reverse	10252 sequences	0.0001%*
	Probe	10252 sequences	100%

*99.99% of sequences (10251 sequences) have a single mismatch in the binding region for the RdRP reverse primer. It was confirmed that one mismatch discovered by *in silico* analysis in the binding region of RdRP reverse primer has no effect on the performance of the PowerChek™ 2019-nCoV Real-time PCR Kit.

In conclusion, none of the analyzed sequences showed mismatches in more than one oligonucleotide and none of the mismatched sequences showed mismatches with both specific assays (E gene and RdRP gene), hence the reactivity of the PowerChek™ 2019-nCoV Real-time PCR Kit is unlikely to be affected.

- **Cross-reactivity**

***In silico* analysis**

Potential cross-reactivity was assessed through *in silico* analysis of each primer and probe sequence against other similar human respiratory pathogens (Table 17). No potential unintended cross-reactivity to other pathogens except for SARS coronavirus is expected. The binding sites for the E gene primers and probe are 100% homologous to SARS coronavirus indicating that

amplification of the E gene target can occur in samples from patients infected with other Sarbecovirus members including SARS, SARS-CoV-2, and SARS-related viruses. There was greater than 80% homology of the RdRP primers and probe to SARS coronavirus, but detection is not expected and this was confirmed in laboratory testing.

Table 17. Strains used for *in silico* cross-reactivity

Other high priority pathogens from the same genetic family	**High priority organisms likely in the circulating area**
Human Coronavirus OC43	Adenovirus 71
Human Coronavirus 229E	Human Metapneumovirus
Human Coronavirus NL63	Influenza A
SARs Coronavirus	Influenza B
MERS-CoV	Influenza C
	Enterovirus D68
	Respiratory syncytial virus A
	Respiratory syncytial virus B
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius

Cross-reactivity wet testing

To evaluate the analytical specificity of the PowerChek™ 2019-nCoV Real-time PCR Kit with regards to cross-reactivity, genomic RNA or DNA from different relevant pathogens was used for testing with the PowerChek™ 2019-nCoV Real-time PCR Kit. Organisms including 23 viruses and 4 bacterial strains were spiked in viral transport media at 10^4 – 10^5 copies/µL and 10^6 CFU/mL, respectively along with the RNA Process Control. Each spiked sample was extracted using the QIAamp DSP kit and tested in triplicate with the PowerChek™ 2019-nCoV Real-time PCR Kit on the Applied Biosystems™ 7500 Real-time PCR System. No cross-reactivity of the PowerChek™ 2019-nCoV Real-time PCR Kit with genomic RNA or DNA of the selected pathogens was observed except for the expected detection of SARS-CoV by the E gene primers and probe. All samples generated a positive Internal Control signal in the JOE channel, whereas no signal was observed in the target-specific (FAM) channel (Table 18).

Table 18. Cross-reactivity test results

Pathogen	E gene	RdRP gene
Human Coronavirus OC43	Negative	Negative
Human Coronavirus 229E	Negative	Negative
Human Coronavirus NL63	Negative	Negative
SARS Coronavirus	Positive	Negative
MERS-CoV	Negative	Negative
Influenza A (H1N1)	Negative	Negative
Influenza A (H3)	Negative	Negative
Influenza A (H5)	Negative	Negative
Influenza B	Negative	Negative
Adenovirus	Negative	Negative
Parainfluenza virus 1	Negative	Negative
Parainfluenza virus 2	Negative	Negative
Parainfluenza virus 3	Negative	Negative
Respiratory syncytial virus subtype A	Negative	Negative
Respiratory syncytial virus subtype B	Negative	Negative
Enterovirus D68	Negative	Negative
Human Rhinovirus	Negative	Negative
Coxsackie A6	Negative	Negative
Echovirus 5	Negative	Negative

Enterovirus 71	Negative	Negative
Streptococcus pneumoniae	Negative	Negative
Haemophilus Influenzae	Negative	Negative
Mycoplasma pneumoniae	Negative	Negative
Legionella pneumophila	Negative	Negative
Rubella virus	Negative	Negative
Mumps virus	Negative	Negative
Measles virus	Negative	Negative

- **FDA SARS-CoV-2 Reference Panel Testing**

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the QIAamp® DSP Viral RNA Mini Kit (QIAGEN, Cat. No./ID: 60704) and the Applied Biosystems™ 7500 Real-time PCR System. The results are summarized in Table 19.

Table 19. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	nasopharyngeal swab (NPS)	5,400 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL =RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

- **Clinical evaluation**

Clinical study using the contrived clinical samples

A contrived clinical study was performed to evaluate the performance of the PowerChek™ 2019-nCoV Real-time PCR Kit. 80 negatives and 60 contrived positives were tested. The viral genomic RNA of SARS-CoV-2 (Human coronavirus (BetaCoV/Korea/KCDC03/2020), NCCP43326) was obtained from the National Cell Collection for Pathogens (NCCP).

The lysis buffer from QIAamp® DSP Viral RNA Mini Kit (QIAGEN, Cat. No./ID: 60704) was prepared by adding the RNA process control (Kogenebiotech, IC0002) before preparing the contrived clinical samples. The contrived positive clinical samples were prepared by spiking the SARS-CoV-2 genomic RNA into individual negative clinical nasopharyngeal swab and sputum samples to approximately 2X LoD (20 samples), 3X LoD (5 samples), and 5X LoD (5 samples). Contrived samples were blinded, randomized, extracted using the QIAamp® DSP Viral RNA Mini Kit, and then tested with the PowerChek™ 2019-nCoV Real-time PCR Kit on the Applied Biosystems™ 7500 Real-time PCR System.

All contrived positive samples generated the expected results for the assay's targets and all negative samples were non-reactive and negative for all assay targets. Result is shown in Table 20 and 21.

Table 20. Clinical evaluation study - Nasopharyngeal swabs

Sample Concentration	Number of positives	Mean Ct	
		E gene	RdRP gene
2X LoD	20/20	34.44	36.38
3X LoD	5/5	33.46	34.60
5X LoD	5/5	32.12	33.61
Negative	0/50	nd	nd

Positive percent agreement: 30/30 = 100% (95% CI 88.43-100.00%)

Negative percent agreement: 50/50 = 100% (95% CI 92.89-100.00%)

nd – not detected

Table 21. Clinical evaluation study - Sputa

Sample Concentration	Number of positives	Mean Ct	
		E gene	RdRP gene
2X LoD	20/20	35.17	36.17
3X LoD	5/5	34.04	34.75
5X LoD	5/5	32.90	33.83
Negative	0/30	nd	nd

Positive percent agreement: 30/30 = 100% (95% CI 88.43-100.00%)

Negative percent agreement: 30/30 = 100% (95% CI 88.43-100.00%)

nd – not detected

Clinical study using clinical samples previously tested by another EUA RT-PCR authorized assay

Performance of the PowerChek™ 2019-nCoV Real-time PCR Kit was evaluated using individual upper and lower respiratory clinical specimens previously tested from April to June 2020. In total, 30 positive (15 nasopharyngeal swabs and 15 sputa) and 30 negative (15 nasopharyngeal swabs and 15 sputa) clinical samples were tested for clinical validation of the PowerChek™ 2019-nCoV Real-time PCR Kit in comparison to an FDA authorized comparator assay.

The randomized, blinded samples were tested with the PowerChek™ 2019-nCoV Real-time PCR Kit on the Applied Biosystems™ 7500 Real-time PCR System and the FDA authorized comparator assay. Performance of the PowerChek™ 2019-nCoV Real-time PCR Kit demonstrated 100% PPA and 100% NPA in comparison to the FDA authorized comparator assay (Table 22).

Table 22. The clinical evaluation results of the PowerChek™ 2019-nCoV Real-time PCR Kit

			Comparator assay				
			Positive		Negative		Total
			NPS	Sputum	NPS	Sputum	
PowerChek™ 2019-nCoV Real-time PCR Kit	Positive	NPS	15	0	0	0	15
		Sputum	0	15	0	0	15
	Negative	NPS	0	0	15	0	15
		Sputum	0	0	0	15	15
	Total		15	15	15	15	60
Positive percent agreement (PPA)			30/30; 100% (95% CI 88.43-100.00%)				
Negative percent agreement (NPA)			30/30; 100% (95% CI 88.43-100.00%)				

16. Explanation of symbols used in Packaging

Symbol	Explanation
	Catalog Number
	In vitro diagnostic use
	Authorized representative in the European Community
	Date of Manufacture
	Manufactured by
	Batch code
	Contains sufficient for <n> tests
	Not to be used in case package is damaged
	Attention. See instruction for use
	Temperature Limitation
	Negative control
	Positive control
	Use by
	Keep away from sunlight

17. Customer and technical supports

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