PLEASE READ THIS PACKAGE INSERT CAREFULLY BEFORE USE

For in vitro diagnostic use only

Prepared: June 2021 (1st version)

SARS Coronavirus nucleic acid detection kit

KANEKA Direct RT-PCR kit SARS-CoV-2

[Important basic precautions]

1. Negative results from this test do not preclude SARS-CoV-2 infection.

2. Results from this test should not be used as the sole basis to diagnose SARS-CoV-2 infection; infection status should be determined comprehensively by including the patient's clinical symptoms. Refer to the updated information for medical institutions and inspection agencies released by the Ministry of Health, Labour and Welfare.

3. Use the necessary biohazard measures when collecting and handling specimens.

4. For specimens to be used for testing, please refer to the "Guidelines for Novel Coronavirus Infection (COVID-19) Pathogen Testing" published by the Ministry of Health, Labour and Welfare.

[General precautions]

1. KANEKA Direct RT-PCR kit SARS-CoV-2 (the Product) is an in vitro diagnostic Product. The Product should not be used for any intended uses other than that described in this Package Insert.
2. The Product should be used in accordance with the intended use and operation method described in this Package Insert. Performance is not guaranteed if the Product is used for any purpose or by any operating method not described in this Package Insert.
3. Always wear protective clothing, masks, safety glasses, gloves, etc. when using the Product, and take care to prevent the reagents from contacting your eyes, skin, and mucosa. If such contact occurs, rinse thoroughly with plenty of water and consult a physician as necessary.
4. Regarding instruments and equipment to be used, please read this Package Insert or the Instructions for Use. They must have been properly serviced and calibrated.
5. When using the Product, this test should be performed under the guidance of personnel who are proficient in performing virus and bacteria testing.

[Configuration and structure (Kit content)]

1. RNA Extraction Reagent: 400 µL, 1 vial (for 100 tests)
2. RT-PCR Enzyme Mix: 600 µL, 1 vial (for 100 tests)
   Reverse transcriptase
   DNA polymerase
   Deoxyribonucleoside triphosphate
3. Primers & Probes Mix: 500 µL, 1 vial (for 100 tests)
   2019-nCoV-N1 F-Primer
   2019-nCoV-N1 R-Primer
   2019-nCoV-N1 Probe
   2019-nCoV-N2 F-Primer
   2019-nCoV-N2 R-Primer
   2019-nCoV-N2 Probe
   IC F-Primer
   IC R-Primer
   IC Probe
   IC Template DNA
4. RNase Free Water: 900 µL, 1 vial (for 100 tests)

[Intended use]

For detection of SARS-CoV-2 RNA in biological samples
(To help diagnose SARS-CoV-2 infection)

(Precautions for use related to the intended use)

Read the sections [Precautions for operation] and (Clinical performance test results) to familiarize yourself with the contents, and be sure to understand the benefit of the Product before selecting the specimen samples.

[Test principle]

The Product uses reagents to detect SARS-CoV-2 genes contained in biological samples by 1-step RT-PCR (the Reverse Transcription Polymerase Chain Reaction method) with a fluorogenic probe. The Product's Primers & Probes Mix includes primers and fluorogenic probes consisting of the sequence specific to SARS-CoV-2 RNA, and also primers and fluorogenic probes consisting of internal control (IC) DNA and their specific sequences to confirm that the PCR amplification step is working properly. Regarding the primers and probes for detecting SARS-CoV-2, the Product utilizes the sequences described in "2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes (Effective: 24 Jan 2020)," issued by the US Centers for Disease Control (CDC).

The Product can extract RNA contained in biological samples by mixing biological samples and the Product's RNA Extraction Reagents, and heating the mixture. After using reverse transcriptase to synthesize cDNA from RNA, it amplifies the target sequence in the cDNA with DNA polymerase. The fluorogenic probe contained in the reaction solution is labelled with quencher at the 3'-terminal side and with fluorescent material (for detecting SARS, FAM; for detecting IC, ROX) at the 5'-terminal side. During the process of DNA elongation process that incorporates four types of deoxyribonucleoside triphosphate, the fluorogenic probe decomposes, and the fluorescent material previously quenched by the quencher is released, producing fluorescence. The fluorescence intensity is measured at each PCR cycle by the real-time PCR system. The presence or absence of SARS-CoV-2 RNA in each sample is determined based on the Ct value of the FAM fluorescence of the probe for detecting SARS-CoV-2 and the Ct value of the ROX fluorescence of the probe for detecting IC.

[Precautions for operation]

1. Specimen collection

   To collect and transport each patient's specimen, please refer to the "Guidelines for Novel Coronavirus Infection (COVID-19) Pathogen Testing" published by the Ministry of Health, Labour and Welfare and the "Manual for Collection/Transportation of Specimens Obtained from Patients Suspected of Having 2019-nCoV (Novel Coronavirus) Infection" by the National Institute of Infectious Diseases (NIID). Improper storage or freezing and thawing may affect the test result.
2. Interfering substances/drugs

   The Product does not purify nucleic acids during the pretreatment step. Therefore, when a specimen is suspended in a solution containing a protein-denaturing agent (such as guanidine) or ethanol, the suspension may affect the PCR reaction. If necessary, confirm in advance the suitability with the Product (such as the presence or absence of PCR inhibition) before use.

   * If the solution contains a protein-denaturing agent (such as guanidine), ethanol, PBS, or high concentration of DTT, it is recommended to perform the test after pretreatment for RNA purification.

3. Cross-reactivity

RNA or DNA from a total of 11 types of viruses and bacteria (commercial product) were examined. All test results were negative, and no cross-reactivity was observed.

Types of viruses and bacteria	Evaluation result
Influenza A H1	Negative
Influenza A H5	Negative
Influenza B	Negative
Human coronavirus OC43	Negative
Respiratory Syncytial Virus A	Negative
Respiratory Syncytial Virus B	Negative
Mycoplasma pneumoniae	Negative
Streptococcus pneumoniae	Negative
Legionella pneumophila	Negative
Bordetella parapertussis	Negative
Haemophilus influenzae	Negative

In *in silico* analysis using BLAST to predict the cross-reactivity of N1 and N2 primers/probes, no cross-reactivity with pathogens other than SARS-CoV-2 was observed.

Types of viruses and bacteria used for *in silico* analysis (Excerpts)

Types of viruses and bacteria
Human coronavirus
SARS-coronavirus
MERS-coronavirus
Adenovirus
Human Metapneumovirus (hMPV)
Human parainfluenza virus
Influenza A virus
Influenza B
Influenza C
Enterovirus
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B
Rhinovirus
Parechovirus
Bordetella pertussis
Candida albicans
Corynebacterium diphtheriae
Coxiella burnetiid (Q-Fever)
Chlamydophila pneumoniae
Chlamydophila psittaci
Haemophilus influenzae
Legionella (non-pneumophila)
Legionella pneumophila
Leptospira sp.
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Neisseria elongate
Neisseria meningitidis
Pseudomonas aeruginosa
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus salivarius
Streptococcus pneumoniae
Streptococcus pyogenes

4. Coexisting substances

It was confirmed that the coexisting substances shown in the table below had no effect even if contained in test specimens.

Coexisting substances	Acceptable concentration*
Clarithromycin	2 mg/mL
Ceftazidime	2 mg/mL
Levofloxacin	2 mg/mL
Acetaminophen	2 mg/mL
Loxoprofensodium	2 mg/mL

* The maximum concentration that would not cause any problems even when the above substances are present in RT-PCR reaction solution. This has been confirmed experimentally.

[Dosage and administration (Operating method)]

1. Necessary instruments and equipment
   (1) Real-time PCR system (LightCycler 96, manufactured by Roche Diagnostics, or an equivalent device should be used)
   (2) Container for real-time PCR (8 consecutive tubes, 96 well plates, etc.)
   (3) Heating block (limited to blocks that can be set to 95°C)
   (4) Micropipette
   (5) Tip with filter for micropipette
   (6) Microtube (RNase- and DNase-free)
   (7) Desktop centrifuge (For spin-down)
   (8) Vortex mixer
   (9) Positive control [recommended product separately sold by: Nihon Gene Research Laboratories, Inc., SARS-CoV-2 positive control RNA (US CDC Real-time RT-PCR N1/N2 PC mix)]

2. Pretreatment (RNA extraction)
   (1) Mix 16 µL of specimen with 4 µL of RNA Extraction Reagent in a microtube.
   (2) Perform heat treatment with a device such as a heating block at 95°C for 5 minutes.
   * Immediately after heat treatment, add the mixtures to the real-time RT-PCR reaction solution, and start the reaction. If the pretreated solution is to be stored temporarily, place it on ice or at 4°C.

3. Settings for the real-time PCR system
   (1) Set up the following conditions according to the documented procedures for the real-time PCR system to be used.
   i) Detection channel: SARS-CoV-2 FAM, internal control (IC) ROX
   ii) Amount of RT-PCR reaction solution: 25 µL
   iii) Conditions for RT-PCR reaction

Steps	Temperature	Reaction time	Number of cycles	Fluorescence detection
1	52°C	300 sec	1	OFF
2	95°C	20 sec	1	OFF
3	95°C	5 sec	45	OFF
	60°C	30 sec		ON

4. Preparation of Master Mix for real-time RT-PCR

Prepare the Master Mix according to the composition shown in the table below. The amount prepared should be increased 10% above what is actually needed (Number of specimens + control reactions).

Reagent name	Additive amount
RT-PCR Enzyme Mix	6 µL
Primers & Probes Mix	5 µL
RNase Free Water	9 µL
(Total amount)	20 µL

5. Preparation of the RT-PCR reaction solution

<Specimens>

After adding 5 µL of the RNA extract obtained in Step 2 to a tube, add 20 µL of the Master Mix prepared in Step 4 to the tube, and mix by pipetting.

<Control reaction solution>

For proper evaluation of results, prepare a reaction solution that contains a negative control and a positive control (Prepare solution for at least one reaction in each PCR run).

Use the RNase Free Water supplied with the Product as the negative control, and solution prepared at 10^3 copies/µL from commercially available artificially synthesized RNA as the positive control.

After adding 5 µL of negative control or positive control to a tube, add 20 µL of the Master Mix, and mix thoroughly by pipetting.

6. RT-PCR reaction

Start running the reaction under the conditions set in Step 3.

7. Data analysis

Analyze data according to the documented procedures for the analysis software of the real-time PCR system used, and calculate the Ct value.

[How to evaluate the test result]

<Test result of the control reactions>

After data analysis, confirm that the test result of the control reactions met the following requirements. If it did not meet the requirements, retesting is recommended.

	SARS-CoV-2 (FAM)	IC (ROX)
Negative control	Ct > 40 or Not detectable	Ct ≤ 35
Positive control	Ct ≤ 30	Ct ≤ 35

<Test results of specimens>

Determine a positive or negative test result based on the calculated Ct value according to the following decision table.

		SARS-CoV-2 (FAM)	
		≤ 40	>40 or Not detectable
IC (ROX)	≤ 35	Positive	Negative
	>35 or Not detectable	Positive	Retest is recommend

<Precautions for evaluation>

A tested specimen may be negative for any of the following reasons. Therefore, a negative result from this test does not preclude the presence of SARS-CoV-2. The patient's attending physician should make a comprehensive clinical diagnosis based on the patient's clinical symptoms as well as the test result.

1. If viral the load of the specimen is below the minimum detection sensitivity.
2. If a mutation, deletion or insertion of SARS-CoV-2 genes occurred in the region corresponding to the Product's primers/probes.
3. If the shape of an amplification curve is abnormal.

[Clinical significance]

Novel coronavirus infection (COVID-19) is a viral respiratory infection caused by SARS-CoV-2. The virus has been spreading rapidly across the world and many persons infected with the virus have been confirmed since its emergence was identified in Wuhan City, Hubei Province, China in December 2019. Early diagnosis is essential to prevent further spread of the infection, and RT-PCR testing to amplify SARS-CoV-2 genes is being conducted for this purpose. This Product is an in vitro diagnostic product for detecting SARS-CoV-2 genes by the real-time RT-PCR method. The feature of the Product is that it does not require RNA purification because a simple specimen pretreatment method is adopted. Thereby, a test will be completed within about an hour. Furthermore, the Product can detect internal controls simultaneously, and therefore prevent false-negative results that may arise due to PCR inhibition. For the above reasons, the Product will be useful for assisting in the diagnosis of COVID-19.

(Clinical performance test results)

1. Nasopharyngeal swab specimens

By using positive specimens from 10 patients and negative specimens from 15 patients, the Product was compared with the RNA purification method described in the "Manual for the Detection of Pathogen 2019-nCoV Ver. 2.9.1"[2], issued by the National Institute of Infectious Diseases. The comparison test showed a positive agreement rate of 100% and a negative agreement rate of 100%, with a total agreement rate of 100%.

Nasopharyngeal swab (Actual specimens)		NIID method		Total
		Positive	Negative	
The Product	Positive	10	0	10
	Negative	0	15	15
Total		10	15	25

Positive agreement rate: 100% (10/10); Negative agreement rate: 100% (15/15)

2. Salivary specimens

Positive specimens from nasopharyngeal swabs were spiked into negative salivary specimens to prepare simulated specimens. Using these simulated specimens, the Product was compared with the RNA purification method described in the "Manual for the Detection of Pathogen 2019-nCoV Ver. 2.9.1"[2], issued by the National Institute of Infectious Diseases. The comparison test showed a positive agreement rate of 100% and a negative agreement rate of 100%, with a total agreement rate of 100%.

Saliva (Simulated specimens)		NIID method		Total
		Positive	Negative	
The Product	Positive	10	0	10
	Negative	0	15	15
Total		10	15	25

Positive agreement rate: 100% (10/10); Negative agreement rate: 100% (15/15)

[Performance]

1. Performance

(1) Sensitivity

When the positive control specimens (50 copies/reaction) are tested, SARS-CoV-2 genes are detected within 38 cycles, and internal control genes within 35 cycles.

(2) Accuracy

When the positive control specimens (50 copies/reaction) are tested, they yield a positive result. When the negative control specimen is tested, they yield a negative result.

(3) Within-run reproducibility

When control specimens are tested three times within a run, all of the positive control specimens yield a positive result and all of the negative control specimens show a negative result.

(4) Minimum detection sensitivity (Example)

50 copies/reaction

2. Reference material for calibration

Positive control specimens: Two types of artificially synthesized RNA, each containing about 100 bases of SARS-CoV-2 N genes, are mixed for use.

Negative control specimens: Sterile purified water (DNA-, RNA-, and Nuclease-free)

[Precautions for use and handling]

1. Precautions for handling (hazard prevention)

(1) Use the Product after taking measures against biohazards under the guidance of personnel who are proficient at handling viruses and bacteria.

(2) Always wear protective clothing, masks, safety glasses, gloves, etc. when handling the Product.

2. Precautions for use

(1) Do not use the Product in combination with other reagents.

(2) Store the Product in accordance with the storage condition. Sediment may be found when thawed, but this will not affect the quality. Confirm that reagents are completely thawed before use. After use, they should be stored immediately in accordance with the storage condition.

(3) Do not use expired Products.
(4) Do not replenish reagents of different lots. The lot number is printed on the labels of the outer package and the contents.
(5) The reagent may be repeatedly frozen and thawed no more than 10 times.
(6) Spin down before placing the RT-PCR reaction solution in the real-time PCR system.
(7) Ensure that there is no adhesion of RT-PCR reaction solution or air bubbles on the lid and wall surface of the real-time PCR container.
(8) Perform the test by real-time PCR immediately after preparing the RT-PCR reaction solution.

3. Precautions for disposal
(1) All specimens must be handled as if they contain infectious agents, and discarded in accordance with the safety regulations of your institution.
(2) Dispose of the instruments used for tests in accordance with the internal rules of your institution after heat sterilization or other appropriate procedures.
(3) Discard specimens without opening the lid of the reaction container after the PCR reaction. To prevent possible contamination by PCR amplification product, do not autoclave them.
(4) Disposal of containers and other materials used should be in accordance with the Waste Management and Public Cleansing Act and the internal rules of your institution.

[Storage condition and shelf life]

1. Storage condition: −10°C to −30°C
2. Shelf life: 12 months (The expiry date is indicated on the outer package.)

[Packaging unit]

KANEKA Direct RT-PCR Kit SARS-CoV-2 for 100 tests

(For more details on the constituent reagents, please refer to the section [Configuration and structure (Kit content)].

[References]

1. 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes, issued by the US Centers for Disease Control (CDC) (Effective date: 24 Jan 2020)
2. Manual for the Detection of Pathogen 2019-nCoV Ver. 2.9.1, by the National Institute of Infectious Diseases

[Conditions for approval]

1. As extremely limited data was available at the time of approval, an appropriate study to evaluate the clinical performance of the Product is to be performed after marketing.
2. A real-condition stability study is to be performed after marketing.

[Contact information]

For inquiries, contact

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