Instruction Manual: KANEKA Direct RT-qPCR Kit "SARS-CoV-2"

Precautions for use:

- **Wear protective equipment when using this product, (rubber gloves, protective glasses, mask, etc.).**
- **It is the customer's responsibility to judge and use the results obtained with this product. The Company shall not be liable for any damage or loss caused by determining or using the results, whether directly or indirectly.**
- **Please verify the validity of the result obtained by the operation procedure not described in this instruction manual.**
- **This product is a research reagent and may not be used for the purpose of diagnosing disease or supporting it.**

1. Product Overview

This diagnostic test kit for the novel coronavirus (SARS-CoV-2) uses primers and probes that comply with the "2019-Novel Coronavirus (2019-nCoV) Real-Time rRT-PCR Panel Primer and probe (Effective: 24 Jan 2020)" issued by the Centers for Disease Control and Prevention (CDC). A simple prep process that does not require purification and the One-step RT-qPCR method allows detection of SARS-CoV-2 from nasopharyngeal swab and saliva in about 1 hour. This product is co-developed with AVSS Co., Ltd.

2. Product Components/Storage Conditions

Content	Volume (100 tests)	Storage Temp.	Expiration Date
RNA Extraction Reagent	250 µL×1	Frozen (Below -20°C)	1 year from manufacturing
RT-PCR Enzyme Mix※1	625 µL×2		
SARS-CoV-2 Primer & Probe Mix※2	250 µL×1		
RNase Free Water	1 mL×1		
ROX Reference (High)※3	250 µL×1		
ROX Reference (Low)※3	250 µL×1		

* 1. Includes enzymes, substrates, etc.

* 2. Store it in a light-shielded manner since it contains a fluorescently labeled probe.

* 3. Store it in a light-shielded manner since it contains fluorescent substances.

3. Detection Method

A Target RNA is amplified and detected by performing RT-qPCR using a primer that specifically binds to the N gene (N1, N2) of SARS-CoV-2 and applied to the sample pretreatment solution obtained by mixing and heat-treating nasopharyngeal swab or saliva and RNA extraction reagent. Please be noted that N1 and N2 are not identified and are detected as the same item.

4. How to Use

4.1. Equipment and device required in addition to this kit

- Micropipette and tip with filter for micropipette
- Real-time PCR device (device that can detect FAM)
- Heat block
- 0.2 mL Tube (for qPCR)
- Desktop centrifuge
- Positive control (sold separately)

4.2. Preparation of RNA extract

(1) Thaw the RNA Extraction Reagent attached to this product completely at room temperature. After thawing, mix well by overturning and pipetting, and spin down before opening the lid.

(2) Mix 4 µL of sample (nasopharyngeal swab or saliva) and 1 µL of RNA Extraction Reagent *4,5.

(3) Heat at 85 °C for 5 minutes *6.

*4. For sample collection and transportation, refer to the latest version of the "Manual for sample collection and transport of the specimens from patients suspected of 2019-nCoV (Novel Coronavirus) Infection", published by the National Institute of Infectious Diseases, Japan.

*5. Handle the sample with necessary biohazard measures.

*6. Prepare the RT-PCR reaction solution immediately after the heat treatment.

4.3. Preparation of Master Mix

(1) Let the RT-PCR Enzyme Mix stand on ice and thaw it completely *7. Thaw all reagents except RT-PCR Enzyme Mix at room temperature. After thawing, mix well by overturning and pipetting, and spin down before opening the lid.

(2) Prepare the required number+α master mix according to the composition in the table below.

Master mix for 1 reaction（when not applying ROX Reference）

Reagent Name	Volume
RT-PCR Enzyme Mix	12.5 µL
SARS-CoV-2 Primer & Probe Mix	2.5 µL
RNase Free Water	5 µL
(Total Volume)	20 µL

Master mix for 1 reaction（when applying ROX Reference*8）

Reagent Name	Volume
RT-PCR Enzyme Mix	12.5 µL
SARS-CoV-2 Primer & Probe Mix	2.5 µL
ROX Reference (High) /(Low)	2.5 µL
RNase Free Water	2.5 µL
(Total Volume)	20 µL

*7. If turbidity is observed after thawing, mix thoroughly until it is completely dissolved by inversion mixing.

*8. Use ROX Reference for real-time PCR equipment that requires ROX correction.

4.4. Preparation of RT-PCR reaction solution

<Sample>

Add 20 µL of master mix to the tube containing the RNA extract prepared in 4.2 and mix well by pipetting * 9.

* 9. Immediately carry out the RT-qPCR reaction after preparing the reaction solution.

<Control Solution>

Prepare a reaction solution to which a negative control and a positive control are added in order to properly judge the result (prepare at least one reaction for each measurement).

(1) As a negative control, use RNase Free Water included in the kit, and as a positive control, use a commercially available synthetic RNA prepared at 103 copies / µL *10.

(2) Add 20 µL of master mix to a tube containing 5 µL of negative or positive control and mix well by pipetting.

* 10. We have confirmed that the following products can be used as positive controls.

・Twist Synthetic SARS-CoV-2 RNA Control 1（Twist Bioscience）

・New Coronavirus Positive Control RNA (US CDC Real-time RT-PCR N1 / N2 PC mix)

（Nihon Gene Research Laboratories Inc.）

4.5. RT-PCR Reaction

Set the qPCR tube containing the reaction solution prepared in 4.4. in the real-time PCR device and perform the RT-qPCR reaction under the RT-qPCR conditions shown in the table below (using the FAM channel).

	Temp. (°C)	Time (Seconds)	Cycles
STEP 1	52	300	1
STEP 2	95	10	1
STEP 3	95	5	45
	60	30（ Fluorescence detection : FAM）	

5． Methods of Evaluation

After completing the RT-qPCR reaction, check the amplification curve and confirm that the analysis parameters are appropriate, and then calculate the Ct value. Evaluate the result referring to the criteria in the table below.

Control Reaction Results

	Ct Value	Evaluation
Negative control	No detection	Normal
	Detection	Abnormal *11
Positive control	≦30	Normal
	＞30 or no detection	Abnormal *12

* 11. Confirm that the negative control is not detected. If the Ct value is calculated, there is a suspicion of contamination, so decontaminate the equipment and work environment and remeasure.

* 12. Confirm that the Ct value of the positive control is 30 or less. If the Ct value is greater than 30, or if it is not detected, check the device settings and remeasure.

Sample measurement results

Ct Value	Results
No detection	Negative or below detection limit *13
<40	Positive
≧40	Remeasurement recommended *14

*13. Even if it is judged to be below the detection limit, there is a possibility that a low copy of the new coronavirus gene exists.

*14. Remeasurement is recommended when the Ct value ≥ 40. If the same result is obtained by remeasurement, it is recommended to judge it as positive.

6. Precautions for use

- Strictly adhere to the storage conditions and expiration dates described in this instruction manual.
- The specifications of this product are subject to change without notice.
- Follow the instruction specified by the manufacturer for the equipment and devices to be used.
- Change the tip of the micropipette after each use and be careful of contamination. Also, use a micropipette tip with a filter.
- Before and after using this product, clean the test bench with 0.55% sodium hypochlorite aqueous solution, DNA remover, ultraviolet (UV), etc.
- To prevent misjudgment due to contamination, it is recommended that each step of RNA extraction, preparation of RT-PCR reaction solution, and amplification / detection by real-time PCR device be performed in separate areas. Also, do not open or close the reaction tube after amplification.
- Before opening the tube containing the reagent and RNA extract, spin down with a tabletop centrifuge (to avoid scattering of the contents).
- If contamination is confirmed, clean the equipment and devices to be used according to the method specified by the manufacturer.
- When disposing of this product, RT-PCR reaction solution, RNA extract, etc., please dispose of it in consideration of hygiene and environment in accordance with the regulations on waste in the area and the regulations of the facility.
- The performance of this product has been confirmed with the following real-time PCR equipment. If you are using another model, please verify the validity of the detection result.
  LightCycler® 96 System（Roche Diagnostics）

LightCycler® 480 System II（Roche Diagnostics）

QuantStudio 3 Real-Time PCR System（96well,0.2 mL block）（Thermo Fisher Scientific）

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