Emergency use approved product for diagnosis of new coronavirus

LiliF™ COVID-19 Real-time RT-PCR Kit

IVD REF IPH21505.50 Σ 50 -20°C

Emergency use approved product (Approval period : ~2020.00.00)
IBT-QMS-IPH21505.50-R00
RUO (Research use Only)

Development Background

There are four genes in the Coronavirus family. Those are known to alpha, beta, gamma, and delta. Alpha and beta corona viruses can cause illness in both humans and animals, whereas others, such as gamma and delta coronaviruses, only infect animals.

Reported illnesses have ranged from mild cold symptoms by Coronavirus 229E, NL63, OC43, or HKU1 to severe illness (e.g., pneumonia) by MERS-CoV and SARS-CoV. COVID-19 is a new coronavirus that has not previously identified.

The new coronavirus (COVID-19) belongs to beta and is one of the new infectious corona viruses that infects the human body as a pathogen of mass pneumonia that occurred in Wuhan, Hubei, China in December 2019. It is very important to diagnose an infection quickly, because there are no vaccines or antivirals approved for prophylactic or therapeutic purposes.

Accordingly, by detecting the RdRp and E genes that are mainly used in Korea as of February 2020, and the N genes, which are recently used as a standard for testing in the US CDC, the diagnostic reliability is increased, and only template RNA is added so that anyone can use them. Premix type of product can increase the speed, accuracy and convenience of molecular diagnosis of new corona viruses.

Principle

- LiliF™ COVID-19 Real-time RT-PCR Kit can detect the new coronavirus using probe method of Real-time RT-PCR, through the reacting of the specific primer and Fluorescent probe in sample.
- This product is provided with a quantitative aliquot of a reagent, primer and probe that performs real-time RT-PCR easily to detect new coronaviruses. The user can immediately experiment by adding RNA extracted from the sample.
- LiliF™ COVID-19 Real-time RT-PCR Kit can detect RdRp and E gene, markers for detecting new coronaviruses. Also, N gene suggested by the US CDC and RNaseP gene which can confirm the validity of all test reactions are adopted and designed for simultaneous detection.

Instrument

- Real-time PCR Instrument
- Pipettes and Disposable Filter Tips
- Disposable Latex Gloves
- Virus DNA/RNA Extraction kit
- Desktop PCR Tube Centrifuges
- Vortex mixer

Kit Contents

No	Component	Ingredient
1	2X RT-PCR mix	< 0.01% dNTPs (dATP/dTTP,dGTP,dCTP) < 0.01% Hot start Taq DNA Polymerase < 0.01% Reverse Transcriptase,
2	RdRp/E Detection solution	< 0.005% RdRp Primers/probe < 0.005% E Primer/Probe
3	N/RNaseP Detection solution	< 0.005% N Primer/probe < 0.005% RNase P Primer/probe
4	Positive Control	< 0.001% Non-infectious plasmid DNA(microbial) containing bertacoronavirus E gene / COVID-19 RdRp gene / COVID-19 N gene / human RNase P gene sequences
5	DNase/RNase Free Water	No template control, 100% DNase/RNase Free Water

Description

1. 2X RT-PCR Mix : Colorless and transparent liquid in colorless microtube.
2. Detection Solution : Colorless (pale-pink colored) and transparent liquid in dark brown colored amber tube
3. Positive Control : Colorless and transparent liquid in colorless microtube.
4. DNase/RNase Free Water : Colorless and transparent liquid in colorless microtube.

Method of Preservation and Period of Use

No	Component	Method of Preservation	Period of use
1	2X RT-PCR mix	Below -20°C, frozen storage	6 months after opening, Within the validity period of the kit
2	RdRp/E Detection solution	Below -20°C, frozen storage	
3	N/RNaseP Detection solution	Below -20°C, frozen storage	
4	Positive Control	Below -20°C, frozen storage	
5	DNase/RNase Free Water	Below -20°C, frozen storage	

Purpose

Sputum in the lower respiratory tract, Bronchoalveolar lavage fluid (BAL), or nasopharyngeal swab (NS) and oropharyngeal smear (Oropharyngeal swab, OS) simultaneously collected from the upper respiratory tract. *In vitro* diagnostic medical devices that help diagnose new coronavirus infection (COVID-19) by qualitatively detecting RdRP genes, E genes and N genes of the new coronavirus (2019-nCoV) from the sample

* Samples should be limited to the type of sample specified in the "Corona Virus Infection Procedures".

Precautions for Use

1. **This product is an emergency use approval product and the use approval period is up to 2020.00.00.**
2. **This product has not undergone clinical performance evaluation.**
3. **This product is a frozen product, in which each components required for the reaction are mixed into tube and must be kept frozen in an environment below -20°C. Please note that the reactivity of the reagent may be significantly lowered if it continues to staying the room temperate more than 1 hour after thawing.**
4. This product should be used for in vitro diagnosis only and should be used by specialists (including medical personnel).
5. All procedures must be carried out in a clean bench and it is recommended that the clean bench be cleaned with alcohol after use.
6. The experimenter should wear lab coat gloves and masks and always be careful.
7. The specimen contains the risk of causing infection and unknown disease, therefore it should be careful when handling it in order to prevent infection by users and indirect contacts.
8. Do not mix reagents from different lots of this product.
9. Carefully handle the reagents and samples of this product to prevent spraying when opening the container lid and to prevent the reagents and samples from sticking to your mouth by wearing a mask.
10. While handling this product and specimens, do not place instruments that may hurt the user, such as needles or knives, and avoid accidents by not using such instruments.
11. If you want to dispose of suspicious specimens, contaminated test materials and instruments, inactivate them by autoclaving and dispose of them. If you want to disinfect, treat them for 10 ~ 30 minutes using 70% ethanol and 0.5% sodium hypochlorite solution.
12. If the target nucleic acid is high concentrations or inhibitors are present, IPC may not be amplified. Dilute the nucleic acid with sterile water and perform the retest.

Belief & Relief, LiliF Diagnostics MDx Kit
Instruction For Use

LiliF Diagnostics

Dosage and Dose (Sample Preparation and Pretreatment)

※ Sample Preparation / Storage and Transportation

[For more information, please refer to the Guide for Sampling Methods for Identification of Corona Virus (Diagnostic Management Team, National Defense Agency]

1. Specimen: Sputum, Bronchoalveolar lavage fluid (BAL) in the lower respiratory tract, or nasopharyngeal swab (NS) and oropharyngeal swab (OS) taken simultaneously from the upper respiratory tract
2. Sample packaging method: Pack the collected sample in the primary container => secondary container => tertiary container, and check the sealed condition of each container and fill in all the accurate sample information.
3. Sample Transport and Storage
   - The specimen transporter should wear N95 equivalent respirator and gloves, and check the type, sampling time and transport time information of the specimen and report the situation to the Emergency Management Center of the Korea Center for Disease Control and Health and Environment Research Institute.
   - Immediately transport to the laboratory at 4ºC as a sample for virus isolation and genetic testing.
   - If transportation within 72 hours is not possible, store at -80ºC and transport using dry ice.
4. Precautions for Sample Extraction and Transport
   - Assignment of suspected specimen transport
   - Compliant with the Transport Guidelines for Infectious Materials
   - Packed samples should be stored in the trunk of self-driving vehicles (or designated vehicles) to prevent them from shaking, and appropriate personal protective equipment, pollution treatment equipment, disinfectants, tripods, etc. It should be prepared inside the transportation vehicle in case of emergency.

※ Nucleic acid extraction from sample (sample pretreatment)

- Use the appropriate viral nucleic acid extraction kit or automated nucleic acid extraction equipment to extract nucleic acids from the sample.
- Depending on the extraction method or kit, the yield and purification purity of the extracted nucleic acid may differ, which may affect the results of real-time PCR analysis.
- As an automated nucleic acid extraction device, Miracle-AutoXT Nucleic Acid Extraction System (Cat.No. IMC-NC15PLUS) and the corresponding AutoXT PGS DNA / RNA Kit (Cat.No. 17168-48, 17168-96) are recommended. In case of Spin-Column Type, our Patho Gene-spin DNA / RNA Extraction Kit (Cat.No. 17054) is recommended.

Dosage and Dose (Test method)

※ Reagent Preparation Required

1. Preparation of Kit Contents
   - Take out the required quantity before starting the test.
   - Leave it at room temperature to thaw it completely, and do not leave it at room temperature for more than 1 hour. Repeated cold thawing can affect performance.
   - This product should be thawed completely with frozen products and centrifuged lightly before testing with the solution collected at the bottom of the tube.
2. DNase / RNase Free Water (positive control) and Positive Control
   - Before the test, put it on room temperature or ice during 10~15mins. Thaw & mix it lightly, and centrifuge it for testing. Use for positive template control and non template control (NTC) for check whether the reaction solution is working properly.

※ Inspection Process

1. Prepare the tube of each Detection Master Mix as +2 quantity of the number of samples.

⚠ An appropriate number of tubes means the combination of two tubes in the number of samples, which includes a positive control and a negative control. In case of real time PCR, the fluorescent signal is passed through the transparent cap of the PCR tube. Be sure not to label the cap and be able to identify it by a separate way.

Contents	Sample	Sample	Positive	Negative
2X RT-PCR Mix	10 µl	10 µl	10 µl	10 µl
RdRp/E Detection solution	5 µl	-	5 µl	5 µl
N/RNaseP Detection solution	-	5 µl	-	or 5 µl
Template	5 µl	5 µl	-	-
Positive Control	-	-	5 µl	-
DNase/RNase Free Water	-	-	-	5 µl
Total volume	20 µl	20 µl	20 µl	20 µl

2. Add 5 µl of distilled water (NTC), gene (RNA) sample, and positive control to each prepared premix and close the cap of the tube.

- Negative controls use 5µl DNase / RNase Free Water instead of genetic samples, and positive controls use 5µl of positive control DNA samples included in the product.
- Real-time PCR (or Real-time RT-PCR) is very sensitive, therefore contamination can be easily identified in negative controls. Therefore, we recommend that you pay attention to contamination such as the use of a filter tip and a pipette for positive control.

3. Mix the reaction solution evenly and spin down to remove the reaction solution from the tube wall and air bubbles at the bottom.

- Real-time PCR does not label the tubes, so be careful not to mix the tubes in this process.

4. Proceed with PCR according to the program set up as follows.

Step	Cycle	Temp	Time
Reverse transcription and Taq activation	1	50 ºC	30 min.
		95 ºC	10 min.
PCR and signal detection	40	94 ºC	15 sec.
		60 ºC	60 sec.

Channel setting	
RdRp & N gene	FAM
E gene & RNase P (IPC)	HEX*

☞ signal detection section

- This product is dispensed quantitatively to adjust an optically clear low-profile PCR tube, so it can be applied to the following equipment compatible with the tube.
  - Applied Biosystems 7500 Fast Real-time PCR System (Thermo Fisher)
  - CFX96 Real-time PCR Detection System (Bio-RAD)

Analysis and Interpretation of the results

※ Parameter Setting

Instruments	Channel	Baseline Setting		Threshold		Ct Cutoff
		RdRp /E	N / RNaseP	RdRp /E	N / RNaseP	
CFX-96	FAM	3~15	3~15	200	200	> 35
	JOE	3~15	3~15	100	200	> 35
ABI 7500	FAM	3~15	3~15	20,000	20,000	> 35
	JOE	3~15	3~15	10,000	20,000	> 35

⚠ The parameter value for baseline setting is based on the positive control solution. If abnormal signal is seen, the setting value can be adjusted by referring to the manual of each equipment manufacturer.

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LiliF Diagnostics

EXPLANATION OF SYMBOLS

Manufactured by | Attention | Consult Instructions For Use

Manufacturing date | Expire date | Keep away from sunlight

Sufficient for tests | Do not reuse | Storage temperature limitation

LOT Batch number | IVD In vitro diagnostic | REF Product number

Document No. : IBT-QMS-IPH21505-R01
Date : 2020. 02. 23

※ Result Analysis

1. As the result judgment depends on the PCR machine used, it is recommended to refer to the manual of the device. For the criteria for interpreting the results, please refer to 'Parameter Setting'.
2. This product contains positive control. Therefore, the effectiveness of this product can be judged as the normal result by reacting positive control and negative control respectively. You can refer to the Ct values in the table below when evaluating the validity.

Contents	FAM	HEX
Positive Control ; PC	20 ~ 25	20 ~ 25
Negative Control (No Template Control ; NTC)	-	-

3. If abnormal results are obtained within the proper storage environment and shelf life of the product, the manufacturer can request a replacement.
4. The detection of IPC is not a prerequisite during the determination of a positive result of a sample. Dominant amplification of other channels may interfere with the IPC signal, resulting in a decrease or no signal.

※ Result

- Check the Ct value of the result obtained from each sample.
- The Ct value is positive when it is within the cutoff criterion, and negative when it is outside the cutoff.
- The following table is an example of the result judgment. Please refer to the result judgment.

Case	Positive Control	Negative Control	Sample		Sample		Interpretation
			RdRp	E	N	RNaseP	
1	+	-	-	+	-	+	Betacoronavirus Positive
2	+	-	-	+	-	-	
3	+	-	+	+	+	+	COVID-19 Negative
4	+	-	+	+	+	-	
5	+	-	+	+	-	+/-	
6	+	-	-	+	+	+/-	
7	+	-	-	-	-	+	Negative (uninfected)
8	+	-	-	-	-	-	Sample Error (Re-extract)
9	+	+	+/-	+/-	+/-	+/-	Nonconformity Results (Retest)
10	-	+	+/-	+/-	+/-	+/-	
11	-	-	+/-	+/-	+/-	+/-	

- RNaseP in set 2 is an internal control and amplification is confirmed if the RNA extracted from human samples is good. Negative RNaseP when other results are positive does not affect the interpretation of the results, but if both negative and RNaseP are also negative, the extraction yields a low yield or reaction-inhibiting substances. You can suspect it and recommend a retest.

Packing Unit

No	Contents	50 tests/kit
1	2X RT-PCR mix	500 µl x 2 tube
2	RdRp/E Detection solution	250 µ x 1 tube
3	N/RNaseP Detection solution	250 µ x 1 tube
4	Positive Control	150µl, 3 tubes
5	DNase/RNase Free Water (Negative Control)	1 ml x 1 tube

Order Information

No	Product Name	Cat. No.
1	LiliF™ COVID-19 Real-time RT-PCR Kit	IPH21505.50
2	Miracle-AutoXT Nucleic Acid Extraction System	IMC-NC15PLUS
3	AutoXT PGS DNA/RNA Kit	17168-48, 17168-96
4	Patho Gene-spin DNA/RNA Extraction Kit	17154

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LiliF Diagnostics

EXPLANATION OF SYMBOLS

Manufactured by
Attention
Consult Instructions For Use
Manufacturing date
Expire date
Keep away from sunlight
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