LiliF™ COVID-19 Multi Real-time RT-PCR Kit

IVD In Vitro Diagnostic | REF IPH21506 | Σ 100 | -20°C

IVD reagents for infectious disease [3]
MFDS Certificate No. 20-327, (Export only)

Development Background

Coronavirus (CoV) is one of the viruses that cause colds, and is classified into 4 genera. Alpha and beta coronaviruses are known to infect humans and animals, and gamma and delta coronaviruses are known to infect animals.
There have been six types of human-infected coronaviruses so far, including types 229E, OC43, NL63, and HKU1, which cause colds, and SARS-CoV and MERS-CoV, which can cause severe pneumonia. Recently, a novel kind of coronavirus (2019-nCoV ; COVID-19) has been identified,
The novel coronavirus (2019-nCoV) belongs to beta and is one of the new infectious coronaviruses that infect the human body as a pathogen of collective pneumonia in Wuhan, Hubei Province, China in December 2019.
It is very important to quickly diagnose an infection because there are no vaccines or antiviral drugs approved for prophylactic or therapeutic purposes, yet.
Accordingly, in order to increase the speed, accuracy, and convenience of molecular diagnosis for the new coronavirus, a product capable of simultaneously detecting RdRP, N and E genes specific to the new coronavirus was designed.

Principle

- LiliF™ COVID-19 Multi 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.
- LiliF™ COVID-19 Multi Real-time RT-PCR Kit can detect RdRP (2019-nCoV specific) and E (betacoronavirus specific) gene, markers for detecting new coronaviruses. Also, N (2019-nCoV Specific) 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.
- LiliF ™ COVID-19 Multi Real-time RT-PCR Kit is a quadruplex RT-PCR product that can simultaneously detect 3 types of 2019-nCoV related markers and human intrinsic gene (RNaseP) in one tube.

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	Contents	100 Tests
1	2X RT-PCR mix	1100 µl x 1 tube
2	COVID Multi Detection solution	550 µl x 1 tube
3	Positive Control	150 µl x 1 tubes
4	DNase/RNase Free Water (Negative Control)	1 ml x 1 tube

Description

1. 2X RT-PCR mix : Colorless and transparent liquid
2. COVID Multi Detection Solution : Colorless (pale-violet colored) and transparent liquid in a amber tube
3. Positive Control : Colorless and transparent liquid
4. DNase/RNase Free Water : Colorless and transparent liquid

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	12 months from date of manufacture
2	COVID Multi Detection solution		
3	Positive Control		
4	DNase/RNase Free Water		

Intended Use

LiliF™ COVID-19 Multi Real-time RT-PCR Kit is in vitro diagnostic medical device based on real-time reverse transcription PCR method intended for the qualitative detection of nucleic acid from the 2019-nCoV in nasopharyngeal/oropharyngeal swabs and sputa from individuals with signs and symptoms of infection who are suspected of COVID-19

Precautions for Use

1. This product should be used for in vitro test only and should be used by specialists (including medical personnel).
2. All procedures must be carried out in a clean bench and it is recommended that the clean bench be cleaned with alcohol after use.
3. The experimenter should wear lab coat gloves and masks and always be careful.
4. 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.
5. Do not mix reagents from different lots of this product.
6. 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.
7. 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.
8. 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.
9. In case of disposing of suspect specimens, contaminated test materials and instruments, should inactivate them by autoclaving, and if disinfecting, should treat them for 10 to 30 minutes using 70% ethanol and 0.5% sodium hypochlorite solution.

Sample Preparation and Pretreatment

※ 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. 17154) is recommended.

Protocols

※ 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	Positive	NTC
2X RT-PCR mix	10 µl	10 µl	10 µl
COVID Multi Detection solution	5 µl	5 µl	5 µl
Sample	5 µl	-	-
Positive Control	-	5 µl	-
DNase/RNase Free Water	-	-	5 µl
Total volume	20 µl	20 µl	20 µl

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Belief & Relief, LiliF Diagnostics MDx Kit
Quick guide

2. Add 5 μl of Sample (RNA), positive control or distilled water (NTC), 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.

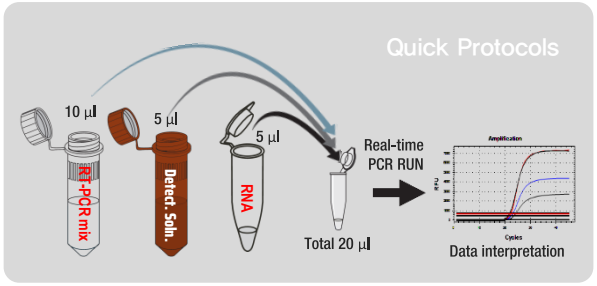
- Real-time PCR does not label the tubes, so be careful not to mix the tubes in this process.

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

Step	Cycle	Temp	Time
Reverse transcription / Taq activation	1	50 ℃	30 min.
		95 ℃	10 min.
PCR and signal detection	40	94 ℃	15 sec.
		*58 ℃	*60 sec.

* signal detection step

Channel setting	
RdRP	FAM
E	HEX, JOE, VIC
N	CAL610, ROX, Texas Red
RNase P	Cy5, Alexa Fluore647



Ordering Information

No	Product Name	Cat. No.
1	LiliF™ COVID-19 Multi Real-time RT-PCR Kit	IPH21506
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, 17154.2

Analysis and Interpretation of the results

※ Parameter Setting

Instruments	Channel	Baseline Setting	Threshold
CFX-96	FAM	3~15 or Auto	200 or Auto
	HEX	3~15 or Auto	200 or Auto
	Texas Red CAL 610	3~15 or Auto	200 or Auto
	Cy5	3~15 or Auto	200 or Auto
ABI 7500 fast	FAM	3~15 or Auto	20,000
	JOE	3~15 or Auto	20,000
	Texas Red	3~15 or Auto	20,000
	Cy5	3~15 or Auto	20,000

⚠ [Cut-off]
- Ct value ≤ 35 : positive, Ct value ＞35 or N/A : negative
- If Ct value of RNase P is "＞35", re-extraction and retesting are required.

⚠ 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.

※ 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	CAL610	Cy5
Positive Control ; PC	20 ~ 25	20 ~ 25	20 ~ 25	20 ~ 25
Negative Control (No Template Control ; NTC)	-	-	-	-

3. 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.

※ Interpretation

- 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 interpretation.

Case	Positive Control	Negative Control	RdRP	E	N	RNaseP	Interpretation
1	+	-	+	+	+	+/-	COVID-19 Detected
2	+	-	+	+	-	+/-	Inconclusive Result*
3	+	-	-	+	+	+/-	
4	+	-	+	-	-	+/-	
5	+	-	-	+	-	+/-	Betacoronavirus positve, but COVID-19 not detected
6	+	-	-	-	-	+	Negative
7	+	-	-	-	-	-	Invalid (Retest)
8	+/-	+	+/-	+/-	+/-	+/-	
9	-	-	+/-	+/-	+/-	+/-	

* Recommended to re-test by increasing the sample concentration. Recommended to proceed with sequencing.

- RNase P used as an internal control is amplified when RNA extracted from human-derived samples is valid. A negative RNase P when other results are positive does not affect the interpretation of the results. However, if RNase P is also confirmed as negative while all are confirmed as negative, the yield of the extraction process may be low, or incorporation of substances that inhibit the reaction may be suspected, and retesting is recommended.

Performance

Criteria	Result
Analytical Specificity	31 DNA/RNA samples were tested on the LiliF™ COVID-19 Multi Real-time RT-PCR Kit in order to evaluate the possibility of cross-reactivity. 31 DNA/RNA samples which have no concern with the detection target of the kit were negative. * Specificity : 100%
Analytical Sensitivity	Serial diluted SARS CoV-2 viral RNA (3 batches, 24 times repeat test each) were tested. * Analytical sensitivity : RdRP : 4.93 x 10^2 copies/test, N : 4.93 x 10^1 copies/test, E : 4.93 x 10^2 copies/test.
Repeatability	Repeatability was confirmed with identical standard substances at different condition; different place, time and person by 3 batch testing. Criteria of repeatability was CV < 1% of Ct value.
Freeze/Thaw Safety	Freeze/thaw safety of LiliF™ COVID-19 Multi Real-time RT-PCR Kit was confirmed by 30 times of Freeze/thaw repeat test. Criteria of safety was CV < 5% of Ct value.

LiliF Diagnostics

EXPLANATION OF SYMBOLS

Manufactured by
Attention
Manufacturing date
Expire date
Keep away from sunlight
Sufficient for tests
Consult Instructions For Use
Storage temperature limitation
LOT Batch number
IVD In vitro diagnostic
REF Product number

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