LiliF™ COVID-19 Real-time RT-PCR Kit

IVD In Vitro Diagnostic | REF IPH21505.50 | Σ 50 | -20℃

Intended Use

LiliF™ COVID-19 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

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, 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 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 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.

Kit contents

No	Contents	50 tests/kit
1	2X RT-PCR mix	1120 µl x 1 tube
2	RdRP/E Detection solution	280 µl x 1 tube
3	N/RNaseP Detection solution	280 µl x 1 tube
4	Positive Control	450 µl, 1 tube
5	DNase/RNase Free Water (Negative Control)	1 ml x 1 tube

[Description]

1. **2X RT-PCR Mix** : Colorless and transparent liquid in colorless micro tube.
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 micro tube.
4. **DNase/RNase Free Water** : Colorless and transparent liquid in colorless micro tube.

Ingredients of Components

Contents	Ingredients
2X RT-PCR mix	< 0.01% Hot start Taq DNA Polymerase, < 0.01% Reverse Transcriptase, < 0.01% dNTPs
RdRP/E Detection solution	< 0.005% RdRP forward primer, < 0.005% RdRP reverse primer < 0.005% RdRP Probe < 0.005% E forward primer, < 0.005% E reverse primer < 0.005% E Probe
N/RNaseP Detection solution	< 0.005% N forward primer, < 0.005% N reverse primer < 0.005% N Probe < 0.005% RNaseP forward primer, < 0.005% RNaseP reverse primer < 0.005% RNaseP Probe
Positive Control	< 0.001% Non-infectious plasmid DNA(microbial) containing partially recombinated RdRP, E, N, RNaseP gene binding sequences
DNase/RNase Free Water	No template control, 100% DNase/RNase Free Water

Storage & Shelf Life

All reagents should be stored at -20 ℃ or below with protection from direct light. The reagents are stable for 12 months when stored in the recommended condition.

Procedure

1. Collecting and Shipping of specimen

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

1) **Collecting specimen** : Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV). https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

Follow specimen collection device manufacturer instructions for proper collection methods.

Swab specimens should be collected using only swabs with a synthetic tip, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

2) **Shipping :** Specimens must be packaged, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Store specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at –70°C ship overnight to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).
https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

2. Preparation before Test

1) Viral RNA Extraction
Detection Kit uses RNA extracted from Sputum, Bronchoalveolar lavage, Oropharyngeal or Nasopharyngeal smears as template for PCR detection. This kit does not include reagents for extracting viral RNA.
The Viral RNA Extraction reagents have different names and characteristics for each manufacturer, the reagents should be used following the manufacturer's instructions. Experimental result may varied depended on the extraction methods and reagents.
(Ex, Patho Gene-spin DNA / RNA Extraction Kit (Cat.No. 17154, iNtRON Biotechnology), QIAamp Viral RNA Mini Kit (Cat. No. 52904, QIAGEN))

2) Recommended instrument

Model	Manufacturer
CFX96™Real-time PCR Detection System	Bio-Rad
ABI 7500 Fast Real-time PCR System	Thermo

3. Preparation of rRT-PCR reaction

1) Prepare the PCR mixture according to the following table.

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	
	RdRP/E	N/RNaseP	RdRP/E	N/RNaseP	RdRP/E	N/RNaseP
2X RT-PCR	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
RdRP/E Soln.	5 µl	-	5 µl	-	5 µl	-
N/RNaseP Soln.	-	5 µl	-	5 µl	-	5 µl
Sample	5 µl	5 µl	-	-	-	-
Positive Control	-	-	5 µl	5 µl	-	-
DNase/RNase Free Water	-	-	-	-	5 µl	5 µl
Total volume	20 µl	20 µl	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.

[Cycling Condition]

Temperature	Times	Cycles
50 ºC	30 min.	1 cycle
95 ºC	10 min.	1 cycle
95 ºC	15 sec.	40 cycles
60 ºC**	60 sec.**	

**Collect fluorescence signal for FAM, and HEX

[Fluorescence Channel Setting]

Channel setting	
RdRP & N gene	FAM
E gene & RNase P (IPC)	HEX (JOE, VIC)

4. Result analysis

1) Reconfirm the designation of fluorophore.
2) Set threshold the following table.
[Parameter Setting]

Instruments	Channel	Baseline Setting		Threshold	
		RdRP/E	N/RNaseP	RdRP/E	N/RNaseP
CFX-96	FAM	3~15	3~15	200	200
	HEX	3~15	3~15	100	200
ABI 7500	FAM	3~15	3~15	20,000	20,000
	JOE	3~15	3~15	10,000	20,000

3) Check the amplification curve and Ct value.
4) Determine the presence of each genes based on the Ct value according to the follow.
* 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.
* N/A : Not analyzed

5. Precautions for analysis result

This kit cannot exclude the possibility of false positive and false negative results due to various factors, completely. The final diagnosis should not be determined solely by the kit, and should be combined with clinical observation, patient history, and epidemiological information.

6. **Result Interpretation**

Case	Positive Control	Negative Control	Assay1		Assay 2		Interpretation
			RdRP	E	N	RNaseP	
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.

RNaseP in assay 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.

7. Quality control

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 (NTC)	-	-

3) If abnormal results are obtained within the proper storage environment and shelf life of the product, the manufacturer can request a replacement.

Performance Specifications

Criteria	Result
Analytical Specificity	31 DNA/RNA samples were tested on the LiliF™ COVID-19 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 dilutions (1000, 100 copies/test) of COVID-19 RNA (3 batches, 24 times repeat test each) were tested. * Analytical sensitivity : RdRP : 100 copies/test, N : 10 copies/test, E : 100 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 Real-time RT-PCR Kit was confirmed by 8 times of Freeze/thaw repeat test. Criteria of safety was CV <5% of Ct value.

Precautions for handling the product

1. This product is intended for diagnostic use, and shall be used by clinical expert such as clinical pathologist and medical technologist.
2. All product components shall be taken out just before use and shall be stored in a freezer (below –20°C) immediately after use they are exposed as little as possible to the ambient temperature.
3. Do not use reagents beyond the expiration date of this product.
4. Do not mix different lots and components.
5. Refrain from repeatedly freezing and thawing samples and products as they may affect the results.
6. While handling the specimen, beware of infection through skin or inhalation. In case of human exposure, the part shall be immediately cleansed with running tap water and medical attention shall be sought immediately for symptoms including high fever and rashes.
7. If the kit's protective packaging is damaged upon receipt, please contact manufacturer for instructions. Attention should be paid to the "use by" date specified on the pack label and individual tube labels. Dispose of unused kit reagents, human specimens and sealed post-amplification plates according to local, state and federal regulations.

Precautions for storage

1. Store this product at-20 ℃.
2. Store the sample and the product, separately.

Precautions for diagnostic results

1. Do not use it for diagnosis of disease only with the test results obtained using this product.
2. Medical specialists should diagnose based on the results of these tests, clinical findings, and other clinical findings.

PACKAGING UNIT

Cat. No.	Name	Package
IPH21505.50	LiliF™ COVID-19 Real-time RT-PCR Kit	50 tests/kit

Storage and Expiration Date

Component	Status	Condition	Period
2X RT-PCR mix	Unopened	Below -20℃	12 months
	Opened	Below -20℃	Within Expiration date
RdRP/E Detection solution	Unopened	Below -20℃	12 months
	Opened	Below -20℃	Within Expiration date
N/RNaseP Detection solution	Unopened	Below -20℃	12 months
	Opened	Below -20℃	Within Expiration date
Positive Control	Unopened	Below -20℃	12 months
	Opened	Below -20℃	Within Expiration date
DNase/RNase Free Water	Unopened	Below -20℃	12 months
	Opened	Below -20℃	Within Expiration date

Trouble Shooting Guide

Problem	Cause	Solution
Fluorescence signal is not detected in all samples	Error of the PCR reaction	• Review if anything is missing during the preparation process.
	If the storage conditions of the kit are not appropriate, or the expiration date has expired	• Repeat the test after checking the storage conditions and expiration date
Fluorescent signal is low in all samples	If the PCR reagents were not mixed correctly	• Proceed the test after review of PCR mix
	Long storage at room temperature or light	• Dispose the kit.
	If the expiration date has passed	• Check the expiration date of the kit.
Signal detection in Negative Control	If the PCR mixture is contaminated	• Discard the PCR mixture.
	If the experiment place or the tool is contaminated	• Check whether the test site or tool is not contaminated. • Repeat the experiment with new aliquots of all reagents
If there are different results in the same sample	Pipetting error	• Check the pipette
	Cross contamination	• Be careful with DNA splitting and repeat the test.

Symbols

Symbol	Description	Symbol	Description
	Do not reuse	IVD	In vitro diagnostic Medical device
	Use by Synonym for this: Expiry Date	LOT	Batch Number
REF	Catalogue Number		Attention. See Instruction for use
	Storage Temperature Limitation		Keep Away From Sunlight
	Manufactured by		Manufacturing Date
EC REP	Authorized Representative in the European Community	Σ	Contains sufficient for Tests
	Consult Instructions For Use	CE	CE marking

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