LiliF™ COVID-19 Kit RT-PCR (deteccion genes RdRP, E y N)

Descripción

INFORMACIÓN DEL PRODUCTO

Hay cuatro genes en la familia del Coronavirus. Se conocen como alfa, beta, gamma y delta. Los virus corona alfa y beta pueden causar enfermedades tanto en humanos como en animales, mientras que otros, como los coronavirus gamma y delta, sólo infectan a los animales.

El nuevo coronavirus (COVID-19) pertenece a los beta y es uno de los nuevos coronavirus infecciosos que infecta el cuerpo humano como patógeno de la neumonía masiva que se produjo en Wuhan, Hubei (China) en diciembre de 2019. Es muy importante diagnosticar una infección rápidamente, ya que no hay vacunas o antivirales aprobados para fines profilácticos o terapéuticos.

Por consiguiente, al detectar los genes RdRp y E que se utilizan principalmente en Corea a partir de febrero de 2020, y los genes N, que se utilizan recientemente como norma para las pruebas en el CDC de los Estados Unidos, se aumenta la fiabilidad del diagnóstico, y sólo se añade el ARN de plantilla para que cualquiera pueda utilizarlos. El tipo de producto de premezcla puede aumentar la velocidad, la precisión y la conveniencia del diagnóstico molecular de los nuevos virus corona.

Principio

LiliF™ COVID-19 El kit de RT-PCR en tiempo real puede detectar el nuevo coronavirus utilizando el método de la sonda de RT-PCR en tiempo real, a través de la reacción del cebador específico y la sonda fluorescente en la muestra.

Este producto está provisto de una alícuota cuantitativa de un reactivo, cebador y sonda que realiza RT-PCR en tiempo real para detectar nuevos coronavirus. El usuario unicamente debe añadir ARN extraído de la muestra.

LiliF ™ COVID-19 El kit de RT-PCR en tiempo real puede detectar el gen RdRp y el gen E, marcadores para la detección de nuevos coronavirus. Además, se incluye el gen N sugerido por el CDC de los EE.UU. y el gen RNaseP, que puede confirmar la validez de todas las reacciones de prueba, y se diseñan para su detección simultánea.

Uso previsto

Esputo en las vías respiratorias inferiores, líquido de lavado broncoalveolar (BAL) o frotis nasofaríngeo (NS) y frotis orofaríngeo (OS) recogidos simultáneamente en las vías respiratorias superiores. Dispositivos médicos de diagnóstico in vitro que ayudan a diagnosticar la infección por un nuevo coronavirus (COVID-19) mediante la detección cualitativa de los genes RdRP, los genes E y los genes N del nuevo coronavirus (2019-nCoV) de la muestra.

Referencia:

LiliF™ COVID-19 Real-time RT-PCR Kit

Este producto es compatible con los siguientes termocicladores:

- Applied Biosystems 7500
- Fast Real-time PCR system (Thermo Fisher)
- CFX96 Real-time PCR Detection System (Bio-Rad)

Contenido del kit

Contenido 48 Pruebas/equipo

1 tira de detección COVID-19 15µl, 12 tiras (96 tubos)

2 Control Positivo 150µl, 3 tubos

3 Agua libre de DNasa/RNasa (Control Negativo) 1 ml x 1 tubo

MSDS

Protocolo

Folleto

LiliF™ COVID-19 Real-time RT-PCR Kit

RUO Research use only | REF IPH21505.50 | Σ 50 | -20°C

Durviz Diagnóstico e investigación www.durviz.com

IBT-QMS-IPH21505-R00

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.
- 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	Contents	50 tests/kit
1	2X RT-PCR mix	560 µl x 2 tube
2	RdRp/E Detection solution	280 µ x 1 tube
3	N/RNaseP Detection solution	280 µ x 1 tube
4	Positive Control	150 µl, 3 tubes
5	DNase/RNase Free Water (Negative Control)	1 ml x 1 tube

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		
3	N/RNaseP Detection solution		
4	Positive Control		
5	DNase/RNase Free Water		

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* Testing regents that help detect 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 has not undergone clinical performance evaluation.
2. This product should be used for in vitro test only and should be used by specialists (including medical personnel).
3. All procedures must be carried out in a clean bench and it is recommended that the clean bench be cleaned with alcohol after use.
4. The experimenter should wear lab coat gloves and masks and always be careful.
5. 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.
6. Do not mix reagents from different lots of this product.
7. 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.
8. 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.
9. 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.
10. 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.

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 4°C as a sample for virus isolation and genetic testing.
   - If transportation within 72 hours is not possible, store at -80°C 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.

LiliF Diagnostics

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Belief & Relief. LiliF Diagnostics MDx Kit

Instruction For Use

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		Positive		NTC	
	RdRP/E	N/RNP	RdRP/E	N/RNP	RdRP/E	N/RNP
2X RT-PCR Mix	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
RdRp/E Detection soln.	5 µl	-	5 µl	-	5 µl	-
N/RNaseP Detection 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.

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

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.

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

- 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.

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
Storage temperature limitation
Sufficient for tests
Do not reuse
Batch number
In vitro diagnostic
Product number
LOT
IVD
REF

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