CE IVD

PCL COVID19 Speedy RT-PCR

Instruction for use

This Instruction for use must be read carefully prior to use, all steps must be and followed accordingly. The assay results may not be reliable if there are any deviations from the instructions in this package insert.

- For in vitro diagnostic use only
- For professional use only

Product Name

PCL COVID19 Speedy RT-PCR

Introduction

COVID-19 is a respiratory disease caused by a new type of coronavirus (SARS-CoV-2) first identified in December 2019 in Wuhan, China. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath, and more. In severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and death. Coronaviruses are a group of viruses that cause symptoms from the common cold to more severe illnesses such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

Intended Use

The PCL COVID19 Speedy RT-PCR is an *in vitro* diagnostic (IVD) real-time reverse transcription PCR (RT-PCR) medical device for the qualitative detection of SARS-CoV-2 genes in human nasopharyngeal specimens from individuals who are suspected of COVID-19 by their health care provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The PCL COVID19 Speedy RT-PCR is intended for use by qualified personnel and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures.

Principle of the Procedure

The PCL COVID19 Speedy RT-PCR is designed for one-step, qualitative RT-PCR assay for the detection of SARS-COV-2 using real-time detection instruments. This kit diagnoses COVID-19 by detecting amplified products based on the fluorogenic probe-based technology, TaqMan probes. This kit uses the Master Mix, which allows efficient cDNA synthesis and PCR amplification, and the products are detected and monitored in real-time with probes. [Primer+Probe Mixture] contains three primer-probe pairs for amplification of 5' end of N gene for confirmatory, 5' end of E gene for screening, and 5' end of RNase P gene for internal control, and tagging FAM dye, Cy3 dye and HEX dye respectively.

Product Descriptions

Materials provided

The reagents contained in one PCL COVID19 Speedy RT-PCR are sufficient for 100 reactions.

	Components	Description	Volume
1	Master mix	Enzyme mix for one-step RT-PCR	500 µl
2	Primer+Probe Mixture	Primer/Probe mix for N, E and RNase P gene	200 µl
3	Positive Control	Cloned plasmid DNAs of N, E and RNase P gene	100 µl
4	DW	RNase-free water	1 ml

Materials required but not provided

- RT-PCR Instrument: Applied Biosystems® 7500 Real-time PCR or Fast Real-time PCR
- RNA extraction kits: QIAamp Viral RNA Mini Kit (QIAGEN)
- PCR tubes or plates
- Pipettes and pipette tips
- Vortex mixer
- Table-top centrifuge
- Disposable gloves and surgical gowns

Storage and Handling Conditions

- All reagent of the PCL COVID19 Speedy RT-PCR should be stored at –20℃ or below. When stored and handled as directed, the reagents are stable until the expiration date indicated on the kit labels.
- Completely thaw the reagents before use.
- Always check the expiration date prior to use. Do not use expired reagents.
- Avoid multiple freeze/thaw cycles. If the reagent are used intermittently, store the reagents in aliquots.

Warnings and Precautions

- For in vitro diagnostic use only.
- For professional use only. Training is required for tests using this kit and operating the equipment used for this test.
- This instruction for use must be read carefully prior to use, all steps must be and followed accordingly. The assay results may not be reliable if there are any deviations from the instructions in this package insert.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Clean and decontaminate surfaces.
- Wear disposable gloves and masks when handling specimens and reagents to prevent contamination. Change gloves between samples and whenever contamination is suspected.
- Calibrate pipettes routinely for accurate results and to not reuse the pipette tips.
- Prepare and validate instruments as directed in manual of each instrument.
- Do not substitute or mix reagent from different kit lots or from other manufacturers.
- Keep reagent tubes and reactions capped as much as possible.
- Do not use the kit if tube is damaged.
- Reagent should only be handled in a clean area and stored at appropriate temperatures.
- Follow standard precautions. All patient specimens should be considered potentially infectious and handle carefully.
- Training in specimen collection is recommended due to the

importance of specimen quality.
- Handel all specimens as if infectious using safe laboratory procedures according to the national biological safety regulations.
- It is recommended that this kit be used in facility with biosafety level II (or higher) for infectious pathogens.
- Use appropriate personal protective equipment including but not limited to disposable gloves, laboratory coat/gown, and eye protection when handling specimens, reagents, pipettes, and other equipment.
- If any of the reagents come into contact with the skin or eyes, wash the area extensively with water. Consult a doctor if there is a problem.
- Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite.
- All waste including unused reagent and human specimens should be disposed in accordance with applicable national regulations.
- Operators should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.
- RNA viruses in particular show substantial genetic variability. Although efforts were made to design rRT-PCR assays to conserved regions of the viral genomes, variability resulting in mis-matches between the primers and probes and the target sequences can result in diminished assay performance and possible false negative results.

Specimen Collection, Handling and Storage

Specimen type and collection
- Nasopharyngeal swab specimens from individuals with signs and symptoms of infection who are suspected of COVID-19 by health care provider.
- Other specimens have not been tested with the PCL COVID19 Speedy RT-PCR.
- Refer to the CDC guidelines for sample collection and storage. (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html)

Storage and transport
- Swabs should be placed immediately into a tube containing 2-3mL of either viral transport medium (VTM).
- After collection, the specimen should be stored at 2-25°C and tested within 48 hours. If exceed 48 hours, specimens should be transported in dry ice and frozen at -70°C or colder.
- Performance may be affected by prolonged storage of specimens.
- Specimens transport should adhere to local and national instructions for transport of pathogenic material.

Assay Procedure

Nucleic acid extraction
- The assay was validated with QIAamp Viral RNA Mini Kit. Other extraction methods have not been validated.
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- If not tested immediately, store extracted RNA at ≤-70℃ until use. Sensitivity of the assay may decrease if specimens are repeatedly frozen and thawed.

Preparation of the RT-PCR reagents
- Completely thaw the kit reagents before use. After thawing, briefly spin-down them.
- To prevent contamination, prepare reagents in a PCR area separated from sample handling area.
- Do not reuse pipette tips.

① Prepare the master mixture by mixing reagents to get the required volume according to the table below.

※ Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for NC and PC and for pipetting error.

Components	Volume per reaction
Master mix	5 µl
Primer+Probe Mixture	2 µl
DW	8 µl
Total (Master Mixture)	15 µl

② Mix by quick vortex and briefly spin-down the mixture.

③ Aliquot 15 µl of the each master mixture into the appropriate PCR tubes respectively.

④ Add 5 µl of extracted RNA sample, Positive Control (PC) and Negative Control (DW) to the tube containing aliquot of the master mixture.

⑤ Spin-down the tube or plate briefly, and run the PCR reaction immediately on Applied Biosystems® 7500 Real-time PCR or Fast Real-time PCR. See details for instrument set-up below.

RT-PCR instrument set-up
- Prepare an instrument according to the instrument operation manual. PCL COVID19 Speedy RT-PCR has been tested with Applied Biosystems® 7500 Real-time PCR or Fast Real-time PCR.
- Define the thermal profile as table below.

[Standard cycling mode] Applied Biosystems® 7500 Real-time PCR

Step	Temp	Time	Cycle	Acquisition mode
Hold	50℃	30 min	1	
	95℃	10 min	1	
Cycle	95℃	15 sec	40	Data detection
	55℃	1 min		

[Fast cycling mode] Applied Biosystems® 7500 Fast Real-time PCR

Step	Temp	Time	Cycle	Acquisition mode
Hold	50℃	5 min	1	
	95℃	2 min	1	
Cycle	95℃	5 sec	40	Data detection
	55℃	30 sec		

Quality control

The PCR controls below are included in the PCL COVID19 Speedy RT-PCR to ensure the validity of each test run. To achieve accurate assay results, one Positive Control (PC) and one Negative Control (NC) must be tested in each test run.

Positive Control is used to confirm assay validity for amplification and detection. The PC is constructed plasmids encoding N, E and RNase P genes as target sequences. Negative Control included in this product may be used as No Template Control (NTC) to confirm assay validity and absence of contaminants. If the Positive and Negative Control results are invalid, samples should be re-tested.

	N (FAM) Ct	E (Cy3) Ct	IC (HEX) Ct
PC	< 35	< 35	< 35
NC	> 40	> 40	> 40

Interpretation of Results and Reporting

Amplification curve must be verified after the reaction is complete. If the amplification curve crosses the threshold line within 40.00 cycles

(<40.00 Ct), a sample is considered as positive and the result can be interpreted according to the below table. If the controls are invalid, the patient results cannot be interpreted and reported.

Summary of interpretation for patient samples

Case	N (FAM)	E (Cy3)	IC (HEX)	Interpretation
1	+	+	+/-	SARS-CoV-2 Positive
2	+	-	+/-	Inconclusive[1)]
3	-	-	+	SARS-CoV-2 Negative
4	-	+	+	SARS-CoV-2 Negative [2)]
5	-	-	-	Invalid[3)]

1) Repeat test. If the sample shows the same result on the retest, an additional confirmatory test may be conducted.

2) SARS-CoV RNA is detected, but SARS-CoV-2 specific RNA is not detected. Repeat test. If the sample shows the same result on the retest, an additional confirmatory test may be conducted.

3) It is recommended to re-extract RNA and re-test the sample.

※ If the target gene signal is strong, the IC (HEX) may be negative.

※ In case of 1 and 2, report results to healthcare provider and appropriate public health authorities.

Limitations of the Procedure

- This kit is used for the qualitative detection of SARS-CoV-2 RNA from human nasopharyngeal swab specimens. The results do not reflect the viral load in the original samples.
- Performance characteristics have been determined with nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection.
- Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out a bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
- A false-negative result may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport, or handling.

Performance characteristics

Limit of detection

The limit of detection (LoD) of the PCL COVID19 Speedy RT-PCR was determined to be 10 copies/rxn for the N gene and 10 copies/rxn for the E gene by testing 20 replicates of RNA spiked samples.

Instrument	Target	Positive rate	LoD
ABI 7500 Real-time PCR	N gene	19/20	10 copies/rxn
	E gene	19/20	10 copies/rxn
ABI 7500 Fast Real-time PCR	N gene	19/20	10 copies/rxn
	E gene	19/20	10 copies/rxn

Cross-reactivity

No cross-reactivity is detected with the following pathogens over 10^6 copies/rxn; Human coronavirus 229E, OC43, HKU1 and NL63, SARS-coronavirus, MERS-coronavirus, Adenovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Influenza A virus (H1N1), Influenza B virus, Enterovirus D68, Respiratory syncytial virus A, Respiratory syncytial virus B, Rhinovirus, Haemophilus influenzae, Legionella pneumophila, Bordetella pertussis, Mycoplasma pneumoniae and pooled nasal wash

Precision

QC samples including negative and positive for SARS-CoV-2, were tested twice a day in triplicate for ten days (total 60 tests per sample for each target for each target). All tests showed consistent results for all panels with 100% positive/negative agreement, and CV% was less than 5%, affirming repeatability.

QC samples were also tested at two laboratories by two operators at each site, twice a day in triplicate for five days (total 60 tests per sample for each target). All tests showed consistent results for all panels with 100% positive/negative agreement, and CV% was less than 5%, affirming reproducibility between-lot, between-operator, and between-site.

Clinical performance

		Comparator assay	
		Positive	Negative
PCL COVID19 Speedy RT-PCR Kit	Positive	50	0
	Negative	0	50

Positive percent agreement: 100% (95% CI: 92.89% to 100.00%)
Negative percent agreement: 100% (95% CI: 92.89% to 100.00%)

Key to Symbols used

REF	List number
IVD	*In vitro* diagnostic medical device
LOT	Batch code
	Consult instruction for use
Σ 100	Sufficient test for 100
-20℃	Stored at -20 ℃
	Expiration Date
	Caution
	Manufacturer
CE	Conformity European
EC REP	European authorized representative

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