CE IVD

Asan qPCR Test® COVID-19

ASAN

※Caution: Please read the precautions and test procedure in this manual before using.

Product name

Product Name	Novel Corona Virus Real Time PCR Detection Kit
Model Name	Asan qPCR Test® COVID-19
Catalogue Number	AM3477-K, AM3478-K

Manufacturing number and expiration date

Refer to external (packaging box) labeling (Lot, Exp. Date)

Package unit

Self-package unit: Refer to external (package box) labeling

Package unit	Quantity	
	100 Tests/Kit (AM3477-K)	250 Tests/Kit (AM3478-K)
COVID-19 Primer/ Probe Mix	50 Tests(265μL) * 2 /Kit	50 Tests(265μL) * 5 /Kit
Onestep RT qPCR reaction mix	50 Tests(525μL) * 2 /Kit	50 Tests(525μL) * 5 /Kit
Positive Control	100μL/Kit	100μL/Kit
Negative Control	500μL/Kit	500μL/Kit

Purpose of use

Asan qPCR Test® COVID-19 is a real-time reverse transcription polymerase chain reaction method from RNA extracted from sputum, nasopharyngeal swab, oropharyngeal swab, and bronchial alveolar lavage fluid in patients with suspected respiratory infections. It is a medical device for in vitro diagnosis that qualitatively detects genes (E gene and N gene) of new coronavirus (2019-nCoV) using Real-time RT-PCR. It is intended to be used by medical professionals who have read the user manual carefully.

How to use

1) Specimen preparation and storage method

(1) Specimens: sputum, nasopharyngeal swabs, oropharyngeal swabs, bronchial alveolar washing fluid

(2) Specimens can be stored for up to 72 hours when stored refrigerated (2-8℃), and stored at -70℃ or lower for long-term storage. The extracted RNA should be stored below -70℃.

2) Preparation process before test

(1) Take out the kit components to thaw them completely. Vortex and centrifuge the reagent lightly before use.

(2) Place each component in a cooler as they are used. Avoid repeated freeze-thaw cycles of the reagent.

3) Test method

(1) Extraction of viral RNA

To extract RNA from the specimen, a commercially available RNA extraction kit is used, and RNA is extracted by referring to the manufacturer's protocol.

(2) PCR mastermix preparation

Each component is mixed and used as shown in the table below.

* Prepare PCR mixture to be 15μL per reaction based on one specimen. At this time, the required number of tests is calculated according to the number of reactions of the specimen, including the positive and negative controls.

Component name	Volume/Reaction
COVID-19 Primer/Probe mix	5 μL
Onestep RT qPCR reaction mix	10 μL
Viral RNA	5 μL
Total volume	**20 μL**

(3) Dispense 15μL of the PCR mixture mixed into the PCR tube to perform real-time PCR.

(4) Add 5μL of each extracted RNA specimen to the PCR tube where the PCR mixture is dispensed, mix the reaction solution, and lightly centrifuge to proceed the reaction according to the PCR conditions below.

At this time, the positive and negative controls provided in the product should be tested with RNA specimens using 5μL each.

Step	Temp (℃)	Time	Cycle
Reverse transcription(RT)	50	15 min	1
Initial Denaturation	95	5 min	1
Denaturation	95	10 sec	45
Annealing & Extension (Data collection)	60	30 sec	

(5) Real-time PCR equipment configuration and operation method follow the equipment manual, and the using fluorescence information is as follows.

Target	Fluorescent dye	Quencher
COVID-19 N gene	FAM	none
COVID-19 E gene	HEX (JOE)	none
IPC	Cy5	none

* Real-time PCR equipment recommended

① CFX96TM Real-time PCR detection system (BIO-RAD)

② Applied Biosystem™ 7500 Fast Real-time PCR Instrument system (Thermo Fisher Scientific)

※ For Applied Biosystems equipment, set the dye to be used as a manual reference condition to NONE, not ROX.

③ Other Real-time PCR equipment supporting fluorescence of FAM, HEX, Cy5

4) Interpretation of results

(1) After real-time PCR is completed, the curve for gene amplification is normally confirmed according to the software of each equipment, and the result is determined when Ct is confirmed.

(2) In order to interpret the results, both positive control should be identified within Ct35, and negative controls can only be determined when the results are confirmed as Ct40 or higher.

(3) If the result obtained from the sample is less than Ct38, it is judged as positive, and if it is more than Ct40, it is determined as negative.

If a value of Ct38 or higher is less than 40, a re-test should be performed. If the result of the re-test meets the positive criteria, it is positive, and if it is Ct38 or higher, it is negative.

Target	Ct cut-off	Result
SARS-CoV-2 N gene	<38	positive
SARS-CoV-2 E gene	<38	positive
IPC	<35	positive

(4) The determination of each result of the 3 genes used in the experiment is as shown in the table below.

Result Interpretation	FAM	HEX(JOE)	Cy5
	N gene	E gene	IPC
COVID-19 positive	+	+	+/-
COVID-19 presumptive positive	+	-	+/-
COVID-19 presumptive positive	-	+	+/-
nCoV negative	-	-	+
Invalid	-	-	-

Storage method

Store in an airtight container at -20℃, 12 months from the manufacturing date.

After thawing once, it can be stored in the refrigerator for 7 days (2-8℃) and used for re-inspection.

Performance evaluation

1) Limit of Detection(Analytical sensitivity)

LoD, the lowest amount of a target that the assay can detect 95% of the time, was determined. Serial dilutions of inactivated SARS-CoV-2 were tested 8 times a day for 3 days. In result, LoD of N gene and E gene are 4.46 copies/reaction and 3.30 copies/reaction, respectively.

Target gene	N				
Conc. (copies/Rx)	25	12.5	6.25	3.13	1.56
Repeat count	24	24	24	24	24
Detection count	24	24	24	16	4
Total positive rate(%)	100.0	100.0	100.0	66.7	16.7
95% Probit	4.46 copies/reaction				
Target gene	**E**				
Conc. (copies/Rx)	25	12.5	6.25	3.13	1.56
Repeat count	24	24	24	24	24
Detection count	24	24	24	22	4
Total positive rate(%)	100.0	100.0	100.0	91.7	16.7
95% Probit	3.30 copies/reaction				

2) Precision(Repeatability)

For repeatability tests, negative, high, medium, and low positive samples were tested 2 runs a day, 3 times per run, for 10 days with 3 lots by one experimenter. In result, the concordance rates within a test, between tests, between dates, and between lots were 100%, and the CV was all within 5%. In conclusion, the Asan qPCR Test® COVID-19 shows repeatability.

Target gene	Specimen Conc. (copies/Rx)	Mean (Ct)	Variation Coefficient(%)
N	25	33.17	1.34
	12.5	35.11	1.75
	6.25	35.92	1.74
E	25	32.36	2.16
	12.5	34.44	2.11
	6.25	35.14	1.89

3) Precision(Reproducibility)

For reproducibility tests, negative, high, medium, and low positive samples were tested 2 runs a day and 3 times per run for 5 days with 3 equipments by 3 different experimenters in 3 different laboratories. The concordance rate between laboratories was 100%, and the CV was all within 5%. In conclusion, the Asan qPCR Test® COVID-19 shows the reproducibility.

Target gene	Specimen Conc. (copies/Rx)	Mean (Ct)	Variation Coefficient(%)
N	25	32.39	1.19
	12.5	34.60	1.92
	6.25	36.00	1.92
E	25	32.24	2.26
	12.5	33.74	2.61
	6.25	35.21	1.62

4) Cross Reactivity(Analytical specificity)

The cross-reactivity against various microorganisms and viruses that may exist in the specimens. 21 pathogens were tested and it was confirmed that Asan qPCR Test® COVID-19 cross-reacted only with SARS Coronavirus. For SARS Coronavirus, the gene target of this product was negative for the N gene and positive for the E gene.

No.	Pathogen	No.	Pathogen
1	Influenza A H1N1	12	Adenovirus Type 3
2	Influenza A H3	13	Coronavirus NL63
3	Influenza A 2009 H1N1pdm	14	Coronavirus 229E
4	Influenza B	15	Coronavirus OC43
5	Metapneumovirus 8	16	Coronavirus HKU-1
6	Respiratory Syncytial Virus A	17	M.pneumoniae M129
7	Rhinovirus 1A	18	C.pneumoniae CWL-029
8	Parainfluenza virus Type 1	19	B.pertussis A639
9	Parainfluenza virus Type 2	20	MERS-CoV
10	Parainfluenza virus Type 3	21	SARS-CoV
10	Parainfluenza virus Type 4		

5) Interference(Analytical specificity)

The effects of various endogenous and exogenous interfering substances that may exist in the specimen were tested. Negative and positive samples containing interfering substances with specific concentration were tested. In result, Asan qPCR Test® COVID-19 shows no interference with those interfering substances.

No.	Interfering substance	Analytical concentration
1	Mucin	1mg/ml
2	Human whole blood	5%
3	Nasal spray	0.1%

6) Clinical evaluation

To evaluate the clinical performance of the Asan qPCR Test® COVID-19, 60 specimens were tested(15 RNA specimens from positive sputum, 15 RNAs from positive nasopharyngeal and oropharyngeal swabs, 15 RNAs from negative sputum, 15 RNAs from negative nasopharyngeal and oropharyngeal swabs).

The positive percent agreement and negative percent agreement are as follows.

Asan qPCR Test® COVID-19	Confirmation test(EUA)	
	Positive	Negative
Positive	30	0
Negative	0	30
Total	30	30

- Positive percent agreement: 100%(30/30) (95% CI: 88.43% ~ 100%)
- Negative percent agreement: 100%(30/30) (95% CI: 88.43% ~ 100%)

Precautions for use and disposal method

1) Use this reagent only for in vitro diagnosis (for specialist).
2) This reagent is preserved by cryopreservation, and once thawed, repeated freezing and thawing should be avoided. After thawing once, it can be stored in the refrigerator for 7 days and used for re-inspection.
3) When handling specimens, use disposable surgical gloves and be careful as there may be infections due to unknown microorganisms or viruses. Also, wash your hands thoroughly after handling.
4) Medical waste used in the experiment is autoclaved at 121℃ for 1 hour or more and then discarded.
5) Since the possibility of false positive and false negative results cannot be completely excluded due to various factors, the final diagnosis should be made by the expert's judgment according to different test methods and clinical findings.
6) Be careful not to let the reagents of this test come into contact with the skin, eyes, or mucous membranes, and immediately rinse them with plenty of water and get medical diagnosis.
7) The product must be completely thawed on ice, and after thawing, the components must be mixed and centrifuged before use.
8) To prevent false positives and contamination, positive specimens and positive controls are stored separately from this product.
9) Do not use kits beyond the expiration date, and do not mix components of different lots.
10) After the test equipment and test table are finished, cleanly disinfect with 0.5% sodium hypochlorite or an appropriate disinfectant.
11) It is recommended to use a sterile filter pipet tip, and contaminated disposable products should not be reused.

Exchange and Returns

This product has undergone strict quality control. If the expiration date of the product has passed or has been deteriorated, damaged, or contaminated, it will be exchanged.

Symbols for IVD components and reagents

Symbol	Explanation
CE	CE mark
IVD	In-vitro diagnostics Medical devices
LOT	Batch code
	Use by date
Σ	Sufficient for <n> tests
	Consult instructions for use
-20℃	Temperature limit
	Manufacturer
	Manufacture date
EC REP	Authorized representative in the European community

ISO13485:2016

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