In vitro Diganostic medical device

Asan qPCR Test® COVID-19&FluA/B

1. LOT number and Expiry date
Refer to external labelling

2. Intended use
The Asan qPCR Test® COVID-19&FluA/B is a qualitative in vitro diagnostic medical device that helps diagnose SARS-CoV-2 genes (RdRp gene, N gene), Influenza A gene(M gene), and Influenza B gene(NS1 gene) with a nasopharyngeal specimen of a person suspected of having a respiratory infectious disease.

3. How to use
(1) Product description
This product is a One-step RT-PCR product that uses Real-time reverse transcription polymerase chain reaction (Real-time RT-PCR). One-Step RT-PCR uses materials for reverse transcription reaction and PCR reaction mixed in one tube. In this process, cDNA is synthesized from RNA extracted from the specimen in the same tube, and PCR for the target gene is performed from the synthesized cDNA.

The real-time PCR method uses a primer and probe, wherein the amplification product amplified by the primer binds to the probe, the polymerase decomposes the probe, and the fluorescence signal of the generated reporter dye is measured in real time. In this process, the fluorescence signal increases according to the amount of amplification product. The Human RNase P gene, which is an internal positive control (IC), can check the RNA extraction process, whether PCR reaction inhibitors are mixed in the extracted RNA, and whether the sample was properly collected and extracted.

This product simultaneously amplifies the RdRp gene and N gene targeting the new coronavirus (SARS-CoV-2), the M gene targeting the Influenza type A virus, and the NS1 gene targeting the Influenza type B virus. and can be qualitatively detected by measuring the fluorescence signal.

(2) Kit Components
The Asan qPCR Test® COVID-19&FluA/B kit contains sufficient reagents and quality control samples. The kit contains the following:

	Components	Volume	Quantity
1	Onestep qPCR Master Mix	1.1 mL	1 tube
2	Primer/Probe Mix	400 µL	1 tube
3	Positive Control	250 µL	1 tube
4	Negative Control	250 µL	1 tube

※ Material and devices required but not provided
CFX 96™ Real-Time PCR Dectection system(Bio-Rad)
- Nucleic acid extraction system or kit
- Benchtop centrifuge with a rotor for 1.5/2.0 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Appropriate reaction plate or tube
- Pipettes
- Pipette tips with filters
- Disposible latex gloves

(3) Specimen preparation and storage
1) The specimen that can be used is a human nasopharyngeal swab.
2) Specimens stored in transport medium are stable for up to 72 hours in refrigerated storage (2~8℃), and must be stored frozen under -70℃ for long-term storage.
3) Test sensitivity may decrease when samples are stored for a long period of time or are repeatedly frozen and thawed.
※ All clinical specimens are handled as potentially infectious.

4. Procedure
(1) Nucleic acid extraction
It is recommended to use the QIAamp DSP RNA Viral Mini Kit as a nucleic acid extraction reagent, and follow the manufacturer's instructions.

(2) How to test
[Preparation of reagent]
1) Open the box, and thaw each components
2) Lightly voltex each completely melted component and spin-down.

[Mix the components]
1) After considering the number of samples N (number of specimens + Positive Control 1EA + Negative Control 1EA + Extra 1EA) to be tested, prepare 15 ㎕ x N of reaction solution using Onestep qPCR Master Mix and Primer/Probe Mix as shown in the table below.

	Components	1 Test Volume	N Test Volume
1	Onestep qPCR Master Mix	11 ul	11 ul x N
2	Primer/Probe Mix	4 ul	4 ul x N
3	Viral RNA Sample	5 ul	5 ul x N
	Total	20 ul	20 ul x N

2) Voltex the prepared reaction solution lightly, being careful not to create bubbles, and then spin down.
3) Dispense 15 µl of the prepared reaction solution into the PCR tube.
4) Dispense 5 µl of the extracted Viral RNA Sample, Positive Control (PC), and Negative Control (NC) into each PCR Tube. (To minimize contamination, work in the following order: NC > Sample > PC.

[Amplication of Real-time PCR]
1) After dispensing, spin down the PCR tube and place it into the PCR well.
2) Operate the equipment following the user manual instructions.
3) Refer to the RT-PCR equipment settings in the following table.

<Setting of CFX96™ Dx System fluorescent>

Target	SARS-CoV-2 RdRp gene	SARS-CoV-2 N gene	Flu A	Flu B	IC
Fluorophore	CalRed610	Cy5	HEX	FAM	Cy5-5

<Setting of Real-time PCR condition>

	Steps	Temperature	Time	Cycle
1	cDNA synthesis	50℃	5 min	1
2	RTase Inactivation	95℃	3 min	1
3	Pre-Denaturation	95℃	5 sec	5
4	Pre-Annealing/ Extension	60℃	40 sec	
5	Denaturation	95℃	1 sec	40
6	Annealing / Extension	60℃	5 sec	

5. Result analysis

When the test is completed, open the corresponding experiment data in the analysis program, perform the result analysis, check the Cut-off (Ct value) for the target gene, and set the threshold for the IC to 500.

Target	Ct Value	Result
COVID-19 (RdRp gene)	Ct ≤ 36	SARS-CoV-2 RdRp gene positive
COVID-19 (N gene)	Ct ≤ 36	SARS-CoV-2 N gene positive
Flu A	Ct ≤ 36	Influenza A virus positive
Flu B	Ct ≤ 36	Influenza B virus positive
IC	Ct ≤ 34	Internal control positive

Case	SARS-CoV-2 RdRp gene	SARS-CoV-2 N gene	Flu A	Flu B	IC	Interpretation
1	+	+	-	-	+/-	SARS-CoV-2 positive
2	+	-	-	-	+/-	SARS-CoV-2 Inconclusive
3	-	+	-	-	+/-	SARS-CoV-2 Inconclusive
4	-	-	-	-	+/-	Negative
5	-	-	+	-	+/-	Flu A positive
6	-	-	-	+	+/-	Flu B positive
7	-	-	+	+	+/-	Flu A, B positive
8	+	+	+	-	+/-	SARS-CoV-2, Flu A positive
9	+	+	-	+	+/-	SARS-CoV-2, Flu B positive
10	+	+	+	+	+/-	SARS-CoV-2, Flu A, Flu B positive
11	-	-	-	-	+	negative
12	-	-	-	-	-	Invalid/Re-test

- Even if the internal control material (IC) shows a negative result, it may still be judged positive. If each detected gene is strongly positive, the IC result value may be impaired due to the high concentration of the detected gene. Therefore, a positive judgment is made even if the IC is negative.
- SARS-CoV-2 Inconclusive: If all SARS-CoV-2 genes are not positive, a retest is performed.
- Invalid/Re-test: If the test is invalid, a retest is performed.

6. Quality control and validity of results

A minimum of one Negative Control and one Positive Control must be included in each run. All control wells must pass for the Real-time RT-PCR plate to be considered valid.

Target	RdRp gene (CalRed 610)	N gene (Cy5)	Flu A (HEX)	Flu B (FAM)	IC (Cy5-5)
PC	Ct≤36	Ct≤36	Ct≤36	Ct≤36	Ct≤34
NC	N/A	N/A	N/A	N/A	N/A

7. Performance

(1) Analytical sensitivity – Limit of detection (LoD)

To determine the LOD, SARS-CoV-2 (SARS-CoV-2 RNA Control, Vircell), Influenza A Virus, and Influenza B Virus (in vitro transcribed RNA) standards were serially diluted. Each sample was tested 60 times in total, using two lots, with 2 runs per day over 3 days, and 5 tests per run. The lowest concentration at which more than 95% of the total repetitions were positive was confirmed.

Detection gene	Limit of detection (LoD)
	Detection concentration (Copies/μL)
RdRp gene	0.8 Copies/μL
N gene	0.8 Copies/μL
Flu A-H1N1	1.14 Copies/μL
Flu A-H3N2	1.13 Copies/μL
Flu B	2.18 Copies/μL

(2) Precision - Repeatability

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were tested as a set by the same experimenter, twice daily, three times per test, over five days using one lot. The test confirmed that the Ct values detected within each test, between tests, and across test dates had a coefficient of variation (CV) of less than 5%, indicating effective repeatability.

(3) Precision – Reproducibility

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were used as a set to assess reproducibility across locations, testers, and lots. Two testers across two laboratories tested two different lots twice daily for five days, with each test repeated three times. The results consistently showed 100% agreement between negative and positive outcomes, and the coefficient of variation (CV) for detected Ct values was less than 5%. This confirmed effective reproducibility.

(4) Competitive interference

To confirm competitive interference between SARS-CoV-2, Influenza A Virus, and Influenza B Virus analytes, the test was repeated 20 times using one lot of product at each concentration under the specified conditions

The test results confirmed that it met the criteria, and demonstrated that high concentrations (100xLoD) of other targets did not affect the sensitivity of analysis for the target at low concentration (2xLoD).

Reference material	Combination		
SARS-CoV-2	Low concentration (2xLoD)	High concentration (100xLoD)	High concentration (100xLoD)
Influenza A Virus	High concentration (100xLoD)	Low concentration (2xLoD)	High concentration (100xLoD)
Influenza B Virus	High concentration (100xLoD)	High concentration (100xLoD)	Low concentration (2xLoD)

(5) Analytical specificity - cross-reactivity

Forty-four different types of microorganisms and viruses that could be present in samples related to respiratory diseases were selected and tested three times per sample. It was confirmed that there was no cross-reactivity among 44 pathogens.

No	Pathogen name	Concentration	Dection or not
1	Coronavirus OC43	$1.58\times10^{5}TCID_{50}$/mL	N.D.
2	Coronavirus NL63	$1\times10^{5}TCID_{50}$/mL	N.D.
3	Coronavirus 229E	$1\times10^{5}TCID_{50}$/mL	N.D.
4	Human metapenumovirus-3 type B1	$1.25\times10^{5.34}TCID_{50}$/mL	N.D.
5	Parainfluenza virus type 1	$1.14\times10^{8}TCID_{50}$/mL	N.D.
6	Parainfluenza virus type 2	$1\times10^{5}TCID_{50}$/mL	N.D.
7	Parainfluenza virus type 3	$3.21\times10^{7}TCID_{50}$/mL	N.D.
8	Parainfluenza virus type 4A	$1.25\times10^{5.58}TCID_{50}$/mL	N.D.
9	Adenovirus type 1	$3.21\times10^{7}TCID_{50}$/mL	N.D.
10	Adenovirus type 3	$4.75\times10^{5}TCID_{50}$/mL	N.D.
11	Adenovirus type 5	$1.25\times10^{6.53}TCID_{50}$/mL	N.D.
12	Adenovirus type 7a	$1\times10^{5}TCID_{50}$/mL	N.D.
13	Adenovirus type 8	$1\times10^{5}TCID_{50}$/mL	N.D.
14	Adenovirus type 11	$1.25\times10^{6.29}TCID_{50}$/mL	N.D.
15	Influenza A H1N1 Virus	$4.17x10^{5}TCID_{50}$/ml	N.D.
16	Influenza A H1N1pdm09 Virus	13.3 ng/ul	N.D.
17	Influenza A H3N2 Virus	16.4 ng/ul	N.D.
18	Influenza B Virus	12.7 ng/ul	N.D.
19	Influenza B Virus [1A Clade]	13.3 ng/ul	N.D.
20	Respiratory syncytial virus type A	130.1 ng/ul	N.D.
21	Respiratory syncytial virus type B	102.7 ng/ul	N.D.
22	MERS-Coronavirus (Florida/USA-2_Saudi_Arabia_2014)	$1\times10^{5}TCID_{50}$/mL	N.D.
23	Human coronavirus HKU1	$5.5x10^{5}$Copies/ul	N.D.
24	Human SARS Coronavirus	$1.8x10^{4}$Copies/ul	N.D.
25	Human bocavirus	$6.5x10^{5}$Copies/ul	N.D.
26	Rhinovirus B42	$1.31\times10^{5}TCID_{50}$/mL	N.D.
27	Enterovirus B111	$0.7\times10^{4}TCID_{50}$/mL	N.D.
28	*Bordetella pertussis*	$1.227x10^{7}$copies/ul	N.D.
29	*Candida albicans*	$4.519x10^{7}$copies/ul	N.D.
30	*Streptococcus pneumoniae*	$2.318x10^{7}$copies/ul	N.D.
31	*Globicatella sanguinis*	$2.368x10^{7}$copies/ul	N.D.
32	*Klebsiella pneumoniae*	$7.891x10^{6}$copies/ul	N.D.
33	*Mycobacterium goodii*	$8.053x10^{6}$copies/ul	N.D.
34	*Mycobacterium fortuitum*	$6.017x10^{6}$copies/ul	N.D.
35	*Streptococcus pyogenes*	$2.317x10^{7}$copies/ul	N.D.
36	*Staphylococcus aureus*	$1.542x10^{7}$copies/ul	N.D.
37	*Staphylococcus epidermidis*	$1.653x10^{7}$copies/ul	N.D.
38	*Bacillus licheniformis*	$1.302x10^{7}$copies/ul	N.D.
39	*Paenibacillus timonensis*	$1.195x10^{7}$copies/ul	N.D.
40	*Lactobacillus johnsonii*	$2.816x10^{7}$copies/ul	N.D.
41	*Streptococcus salivarius*	$2.383x10^{7}$copies/ul	N.D.
42	*Haemophilus influenzae*	$2.825x10^{7}$copies/ul	N.D.
43	*Haemophilus parainfluenzae*	$2.294x10^{7}$copies/ul	N.D.
44	*Legionella pneumophila*	$1.366x10^{7}$copies/ul	N.D.

N.D: Not detected

(6) Analytical specificity – interference response

Nine types of endogenous or exogenous substances were added to the samples and tested three times per sample. It was confirmed that there was no interference for 9 types of interfering substances up to the corresponding concentration.

No	Interference material	Concentration	Interference or not
1	Saline Nasal spray	10%(V/V)	N.A
2	Zanamivir	3mg/ml	N.A
3	Bilirubin, Conjugated	0.05mg/ml	N.A
4	Albumin	0.24g/ml	N.A
5	Haemoglobin	2mg/ml	N.A
6	Biotin	300ug/ml	N.A
7	Tobramycine	24ug/ml	N.A
8	Mucin	0.1mg/ml	N.A
9	Human genomic DNA	10ug/ml	N.A

N.A: Not available

8. Storage method and period of use

(1) Store the kit in its original sealed packaging at -20℃. or below, away from direct sunlight. Kit contents are stable until the expiration date printed on the outer box.

(2) The number of freeze/thaw cycles is limited to four

9. Precautions

[General precautions]

(1) This product is for in vitro diagnostic use only.

(2) This test should only be conducted by trained professionals.

(3) Before starting the assay, thoroughly read the user manual and follow the instructions for use.

(4) All specimens collected from humans should be considered potentially infectious. Follow Good Laboratory Practice (GLP) when handling samples to prevent contamination.

(5) Avoid contact of this product with skin, eyes, or mucous membranes. If contact occurs, rinse immediately with plenty of water.

(6) Improper control of sample cross-contamination during handling and processing may lead to false positive results.

[Precautions for storage]

(1) Store the product below -20°C.

(2) Do not use products beyond their expiration date.

[Precautions when test]

(1) Before use, visually inspect each component of the product for leaks. Do not use if a leak is detected.

(2) Do not mix this product with products from other lots during use.

(3) Avoid repeated freezing and thawing of the product, as it may affect test results.

(4) This product is optimized for use with the recommended real-time gene amplification device. Use with other equipment may result in incorrect results.

(5) Take care to prevent contamination of product components by nucleic acid extracts, PCR products, or positive controls. We recommend using filter tips to prevent contamination.

(6) In case of spills onto equipment, clean according to the equipment manual and remove contamination from surfaces.

(7) All samples used in testing are potentially infectious. Use appropriate protective equipment and conduct testing in facilities such as biosafety cabinets of Biosafety Level 2 or higher.

(8) Improper collection, transport, or handling of samples may affect test results. Handle samples with caution.

[Precautions when determining results]

(1) Do not utilize test results obtained with this product for disease diagnosis. Diagnosis should be based on test results, clinical findings, and other clinical data evaluated by a healthcare professional.

(2) A negative result alone may not rule out SARS-CoV-2, Flu A, or B infection if the sample's viral concentration is below the detection limit or if there are amplification inhibitors present.

(3) Sensitivity may decrease if there are mutations in the primer and probe binding regions included in the product.

(4) This test does not exclude diseases caused by other bacterial or viral pathogens.

[Precautions for disposal]

(1) Dispose of products and samples following guidelines and methods outlined in the Waste Management Act.

9. Creation and revision date

First creation date : 2024.06.19

[Product name] High-risk infectious agent genetic testing reagent
[Classification number (grade)] K05030.01(3)
[Model name] Asan qPCR Test® COVID-19&FluA/B

그림 다시 첨부