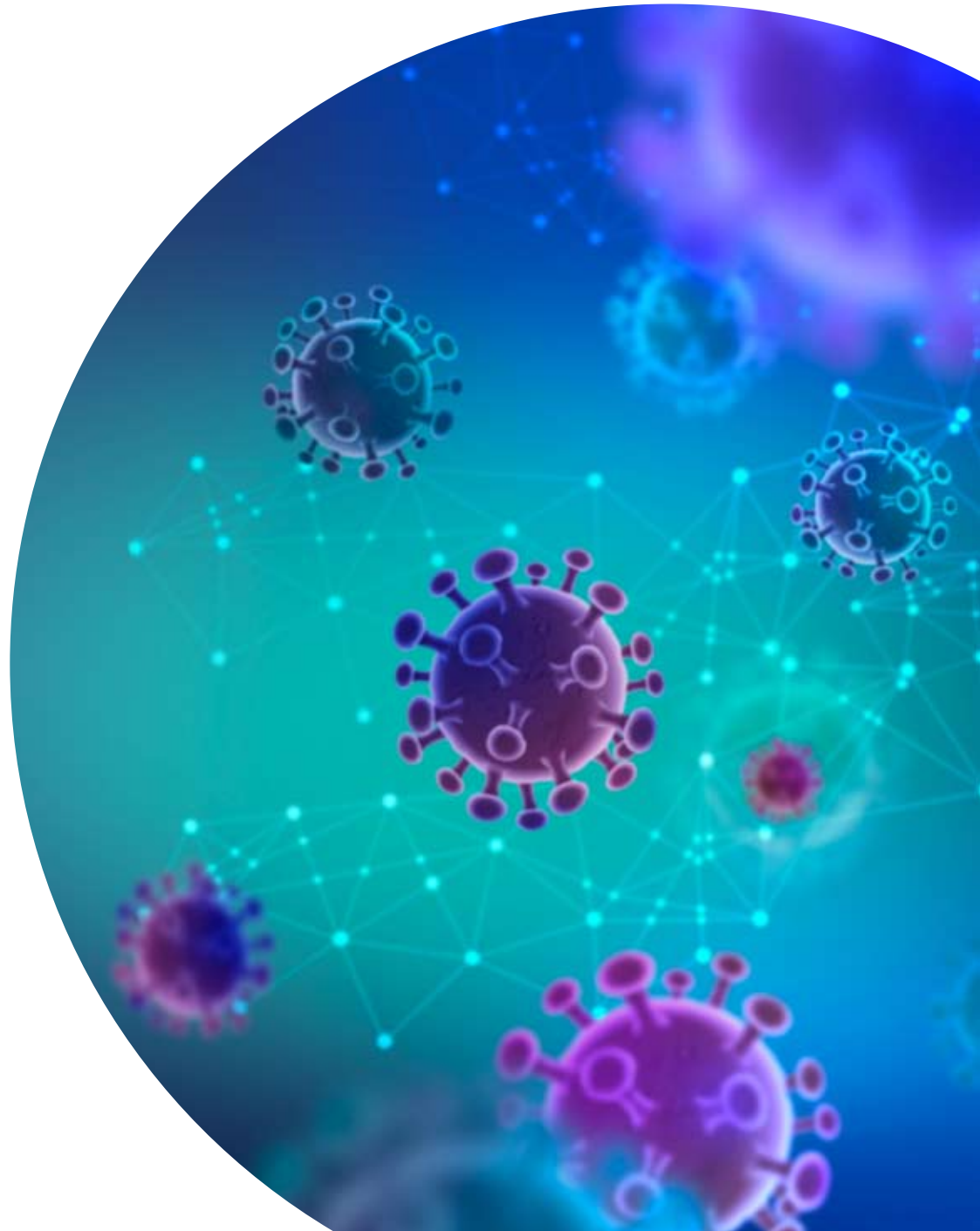




Asan qPCR Test[®] COVID-19

SARS-CoV-2 Real-time RT-PCR Kit

 ASAN PHARM. CO., LTD.



Overview of COVID-19

- **Origin** : December 2019 in Wuhan Hubei Province, China
- **Pathogen** : Severe Acute Respiratory Syndrome - CoronaVirus - 2 (SARS-like CoV-2)
- **Symptom** : Fever, myalgia, diarrhea, cough, skin rash, conjunctivitis, dyspnoea, chest pain, fatigue, sore throat etc.
- **Incubation peorid** : 2 - 15 days

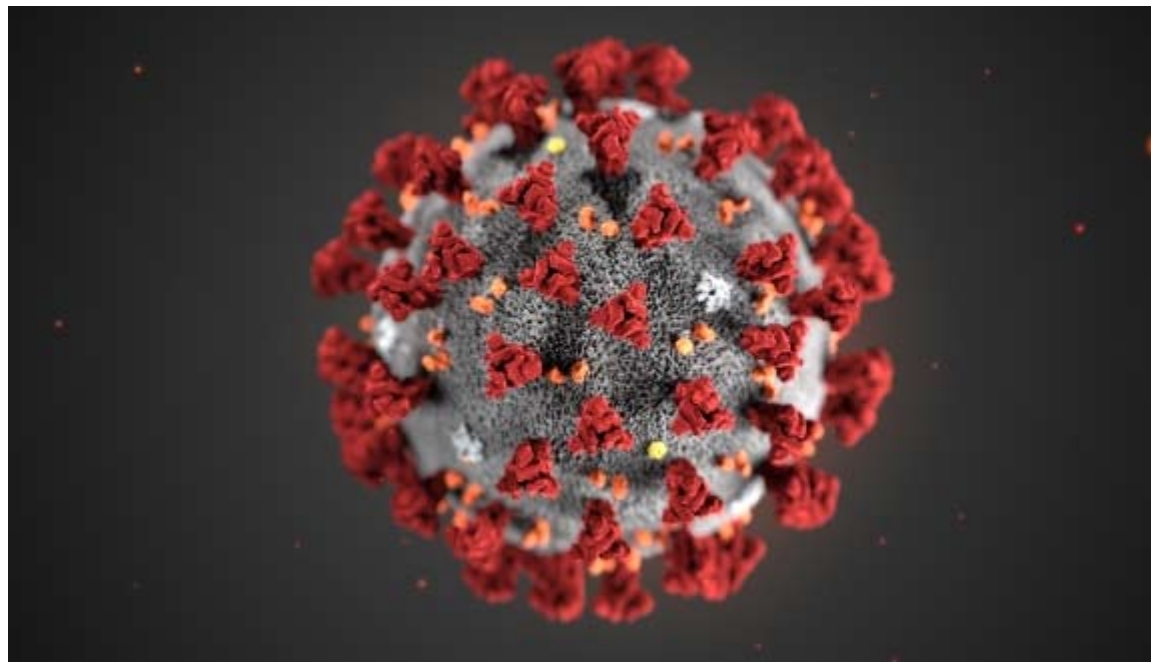
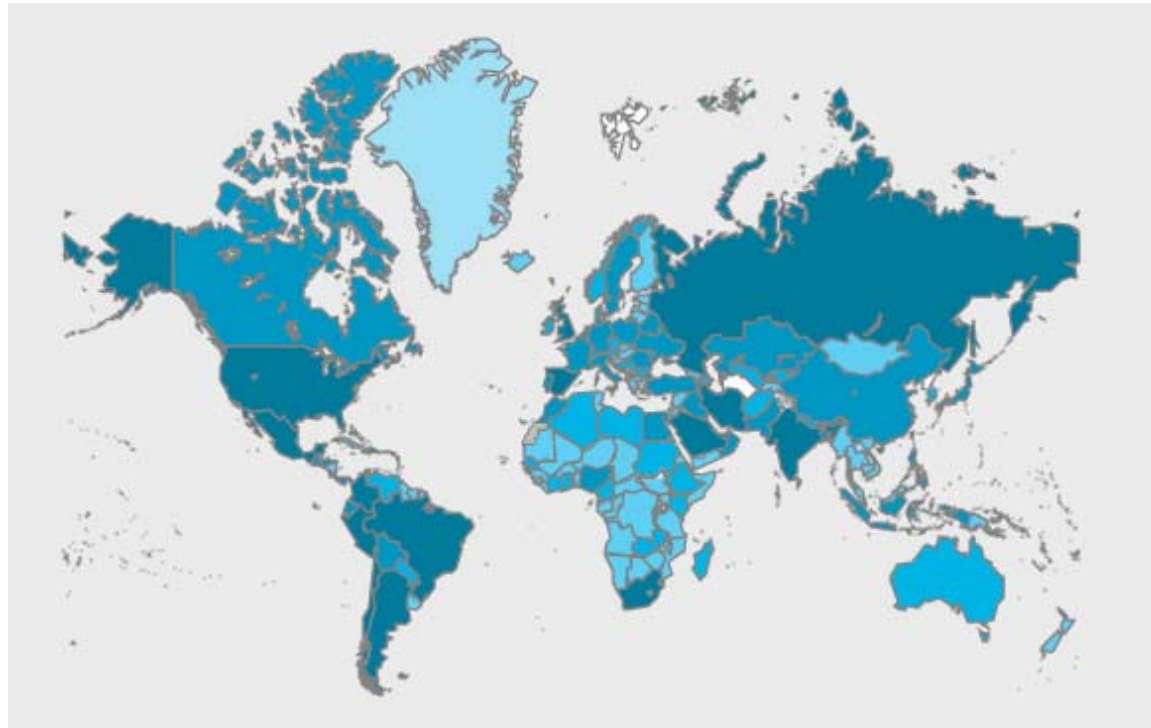


Image reference : Center for Disease Control and Prevention

COVID-19 Outbreak World Map

- 26 August 2020, WHO Coronavirus Disease (COVID-19) Dashboard



- ▶ 23,752,965 confirmed cases
- ▶ 815,038 deaths
- ▶ 216 Countries, areas or territories with cases

Kit Contents



Component name	Quantity (100 Tests/Kit)	Quantity (250 Tests/Kit)
COVID-19 Primer/Probe Mix	50 Tests (265 μ L) \times 2	50 Tests (265 μ L) \times 5
Onestep RT qPCR reaction mix	50 Tests (525 μ L) \times 2	50 Tests (525 μ L) \times 5
Positive Control	100 μ L \times 1	100 μ L \times 1
Negative Control	500 μ L \times 1	500 μ L \times 1

Target gene

- Genetic map of coronavirus and relative positions of amplicon targets on SARS-CoV and Wuhan-CoV genome.

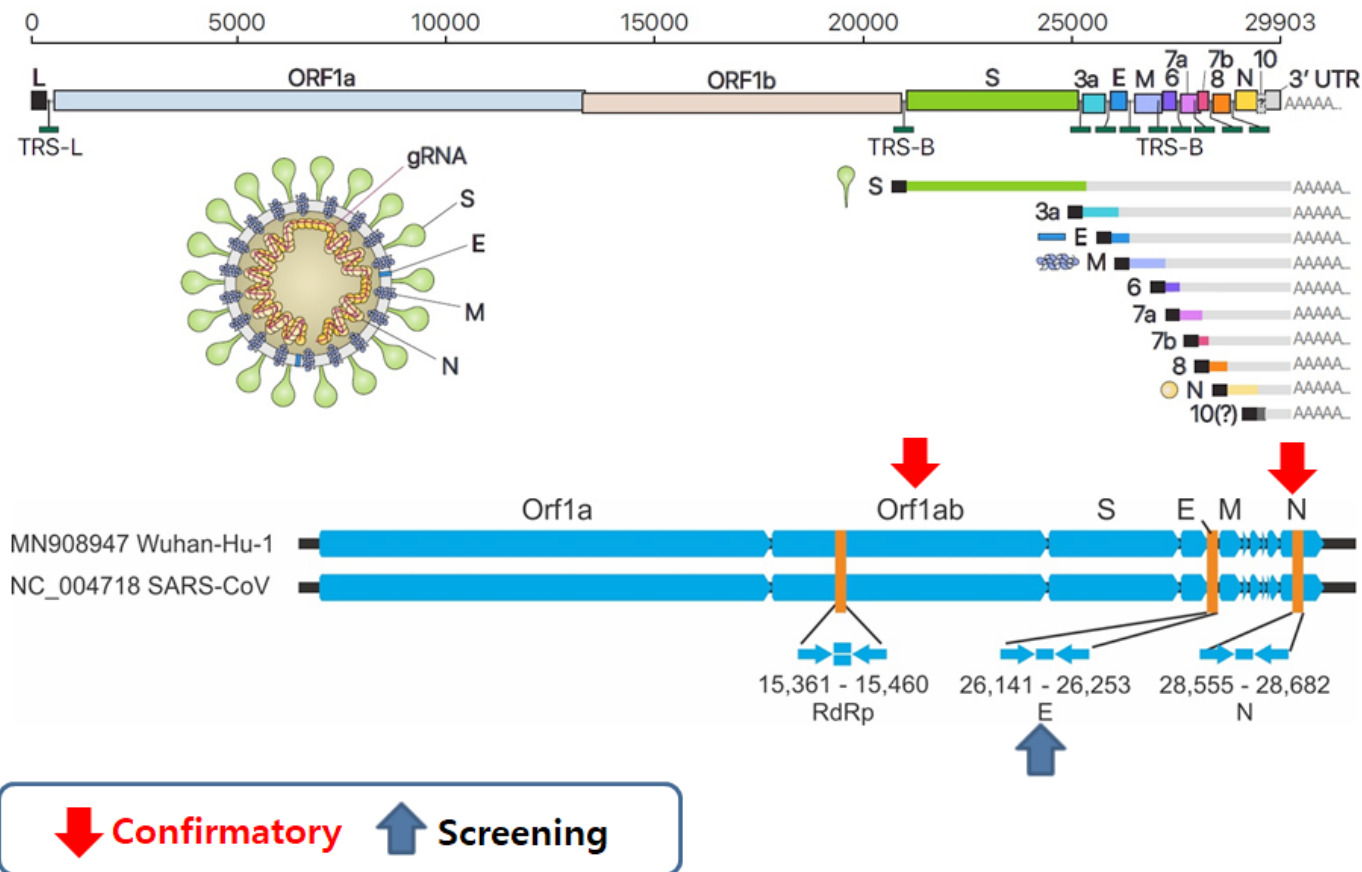


Image reference : Institute for Basic Science & WHO

Target gene

- Common Target Genes for COVID-19 Molecular Diagnosis

E gene : Screening gene for COVID-19

(Reference : Molecular assays to diagnose COVID-19: Summary table of available protocols by WHO guideline, <https://www.who.int/publications/m/item/molecular-assays-to-diagnose-covid-19-summary-table-of-available-protocols>)

N gene : Confirmatory gene for COVID-19

(Reference : Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes by US CDC guideline, <https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html>)

- Asan qPCR Test[®] COVID-19 Fluorescent dye

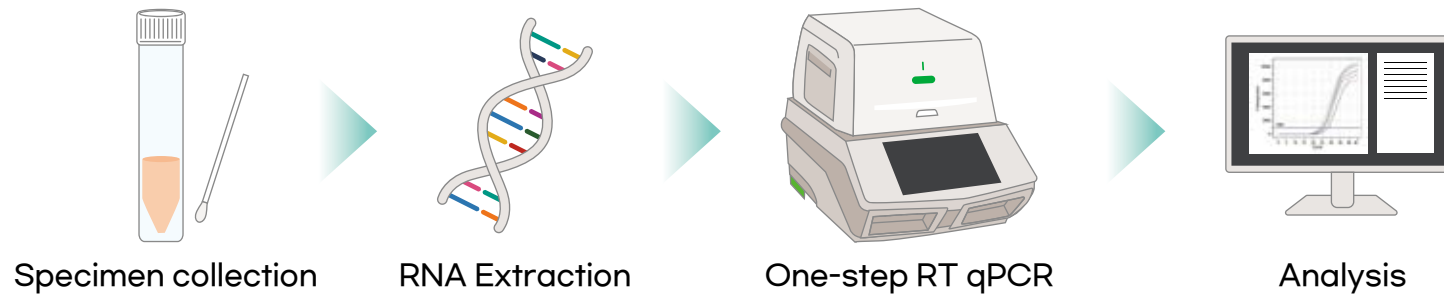
Target	Fluorescent dye	Quencher
COVID-19 N gene	FAM	none
COVID-19 E gene	HEX (JOE)	none
IPC	Cy5	none

Target gene

- Many international molecular diagnostics manufacturers selected nucleocapsid(N) gene as a confirmatory gene including Cepheid, Thermo Fisher, Abbot, GenMark, Perkin Elmer Qiagen, BD and Luminex.

Diagnostic Test Name (Letter of Authorization)	Gene Targets		
CDC 2019-n-CoV Real-Time RT-PCR Diagnostic Panel (CDC)	N1	N2	N3
NewYorkDepartmentofHealth, SARS-CoV-2 Real-time ReverseTranscriptase(RT)-PCR Diagnostic Panel	N	N	
Roche Molecular System Cobas SARS-CoV-2	ORF1ab	E	
Thermo Fisher TagPath COVID-19 Combo Kit	ORF1ab	N	S
LabCorp COVID-19 RT-PCR Test	N		
Quest SARS-CoV-2 rRT-PCR	N1	N3	
Abbot RealTime SARS-CoV-2 assay	N	RdRp	
GenMark ePlex SARS-CoV-2 Test	N		
Cepheid Xpert Xpress SARS-CoV-2 test	N1	E	
Mesa Biotech Accula SARS-CoV-2 Test	N		
PerkinElmer New Coronavirus Nucleic Acid Detection Kit	N	ORF1ab	
AvellinoCov2 test	N1	N3	
Luminex NxTAG CoV Extended Panel Assay	N	ORF1ab	E
Qiagen QiAstat-Dx Respiratory SARS-CoV-2 Panel	RdRp	E	
NeuMoDx SARS-CoV-2 Assay	N	nsp2	
BD BioGX SARS-CoV-2 Reagents for BD MAX System	N1	N2	
ScienceCell SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit	N1	N2	N3
Luminex ARIES SARS-CoV-2 Assay	N	ORF1ab	
BD SARS-CoV-2 Reagents for BD MAX System	N1	N2	
KorvaLabs Curative-Korva SARS-CoV-2 Assay	N1	N2	

Workflow



■ Specimen collection

- Collect samples(Nasopharyngeal & Oropharyngeal swab or Sputum)

■ RNA extraction

- Extraction of nucleic acid by manual or auto extraction method

■ One-step RT qPCR

- Asan qPCR Test[®] COVID-19 & Real time PCR instrument
 - ▶ Reaction proceeds with one tube
 - ▶ Detection of two target genes
 - ▶ Analysis within 2hours after nucleic acid extraction

■ Analysis

- Results analysis by software of each instrument

PCR Mixture preparation

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

Programming of the Real-time PCR instruments

Step	Temp ($^{\circ}$ C)	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	

Analytical sensitivity (Limit of detection, LOD)

- For the detection of COVID-19 RNA, the LOD is 4.46 copies/reaction for N gene and 3.30 copies/reaction for E gene.

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	100	100	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	100	100	91.7	16.7
95% Probit	3.30 copies/reaction				

Ct Cut-off

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

Interpretation of result

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

Analytical specificity

- The Asan qPCR Test COVID-19 did not show with any cross-reaction with the following microorganisms and viruses. However, 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	InfluenzaA 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	MERSV
10	Parainfluenza virus Type 3	21	SARS-CoV
11	Parainfluenza virus Type 4		

Clinical evaluation

- To evaluate the clinical performance of the Asan qPCR Test[®] COVID-19 product, the results of confirming clinical sensitivity and clinical specificity using 60 specimens of the diagnosis of COVID-19 are as follows. (15 RNA specimens from positive sputum, 15 RNAs from positive nasopharyngeal and oropharyngeal swab, 15 RNAs from negative sputum, 15 RNAs from negative nasopharyngeal and oropharyngeal swab)

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

- Clinical sensitivity: 100%(30/30) (95% CI: 88.43% ~ 100%)

- Clinical specificity: 100%(30/30) (95% CI: 88.43% ~ 100%)

Ordering information

Product	Asan qPCR Test [®] COVID-19	
Cat. No.	AM3477-K	AM3478-K
Size	100 tests/kit	250 test/kit
Method	Real-time RT-PCR	
Description	N gene, E gene detection for SARS-CoV-2	

Related product

Product	Cat. No.
Asan Transport Medium	AM608-10
ASANPREP [™] Viral Nucleic Acid Purification Kit	AM3479-K