INSTRUCTIONS FOR USE

A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit

REF RR001

Rx Only

IVD

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Contents

Intended Use

The A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit is an In Vitro Diagnostic(IVD) Real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of SARS-CoV-2 viral nucleic acids in human upper clinical specimens (such as nasopharyngeal and oropharyngeal swab) who are suspected of COVID-19 by their health care provider. Testing is limited to U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

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 infective 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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 A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit is Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.

Principle of the Test

The A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit is based on real-time reverse transcription polymerase chain reaction (RT-qPCR) technology. The Primer and probe of A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit are designed specifically to detect ORF1ab and N genes from SARS-CoV-2 genome in upper respiratory specimens from patients who is suspected of COVID-19.

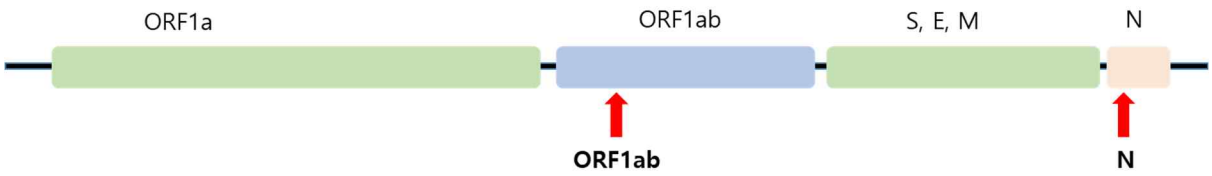


Figure 1. SARS-CoV-2 Genome Structure and

Detection site of "A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit

This kit is designed a multiplex RT-qPCR for detection of SARS-CoV-2 ORF1ab (FAM), N gene (HEX) and Human housekeeping gene RNase P gene (for internal control, Cy5) simultaneously in one tube.

Components and Storage

The A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit must be stored at -24℃ ~ -18℃ until expiration date. Do not use expired reagents from the kit. Do not repeat the freeze/thaw procedure more than 3 times after opening. Exposure to light, heat, or humidity should be avoided due to stability of this kit. If the kit or components have been damaged during transport, please do not use. Keep reagents separate from sample material to avoid contamination.

Table 1. Kit components (100 test/kit)

	Reagent type	Quantity	Capacity (㎕)
1.	4X Master mix	1	500
2	Oligo mix (ORF1ab)	1	300
3	Oligo mix (N)	1	300
4	Oligo mix (Internal control)	1	400
5	Positive control	1	500
6	Negative control (DNase, RNase free water)	1	500

Required materials (Not provided with the kit)

Table 2. Required materials for A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit

	Item	Source
Extraction Instrument and Consumables	KingFisher™ Flex Purification System	Thermo Fisher Scientific Inc (Cat. no. N07669)
	MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher Scientific Inc
	KingFisher Flex 96 tip comb	Thermo Fisher Scientific Inc (Cat. no. 97002534)
	KingFisher 96 KF plate (200 µl)	Thermo Fisher Scientific Inc (Cat. no. 97002540)
	KingFisher 96 Deep-Well Plates	Thermo Fisher Scientific Inc (Cat. no. 95040450)
Real-Time PCR Instrument and Consumables	QuantStudio™5 Real-time PCR System (QuantStudio® Design & Analysis Software v1.4)	Thermo Fisher Scientific Inc (A31671)
	MicroAmp Optical 8-Tube Strip	Applied Biosystems (4358293)
	MicroAmp Optical 8-Cap Strip	Applied Biosystems (4323032)
	MicroAmp Optical Adhesive Film	Applied Biosystems (4311971)
	MicroAmp optical 96-well Reaction Plate	Applied Biosystems (4346907)
	CFX96 Touch™ Real-time PCR Detection System (CFX Manager™ Software v3.1)	Bio-Rad (1855195)
	Low-Profile PCR tubes 8-tube Strip, White	Bio-Rad (TCS0851)
	Optical Flat 8-Cap Strips for 0.2ml tube strips/plate	Bio-Rad (TCS0803)
	Microseal 'B' PCR Plate Sealing Film, adhesive, optical	Bio-Rad (MSB-1001)
	Multiplate 96-Well PCR Plates, low profile, unskirted, white	Bio-Rad (MLL9651)
Consumables and Equipment	Pipettes	MLS*
	Sterilize aerosol barrier (filtered) pipette tips	MLS*
	Disposable powder-free gloves and laboratory coat	MLS*
	Bench top centrifuge	MLS*
	Vortex mixer	MLS*
	Refrigerator	MLS*
	Freezers (-20°C and -80°C)	MLS*
	Sterilize aerosol barrier (filtered) pipette tips	MLS*
	Micro-centrifuge tube	MLS*

* Major Laboratory Suppliers

Warning and Precaution

- The A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit has not been FDA cleared or approved or EUA authorized. Validation of this test has not been reviewed by FDA. The review under the EUA program is pending
- For *in vitro Diagnostic* (IVD) and prescription use only.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Prior to test, all component must be thoroughly thawed, mixed and centrifuged.
- Do not use beyond the expiration date printed on the kit box.
- Keep all the materials on ice when in use.
- Please read the instructions for use carefully before testing.
- All instruments used in the experiment should be sterilized for prevent contamination.
- Do not mix reagents from different lots.
- Discard all materials in a safe and acceptable manner, in compliance with all legal requirements.
- Do not use if the package or any assay components are damaged.
- Keep extracted RNA on cold block or on ice during reaction set-up.
- Avoid possible contamination of reagents with extracted nucleic acids, PCR products, and positive control.
- To prevent contamination of reagents, use of filter-tips is recommended.
- Do not eat, drink or smoke in the area where the specimens and kit contents are handled.

Sample collection, transport, and storage

Sample collection devices are not provided with this kit. For using this kit, upper respiratory (nasopharyngeal (NP) and oropharyngeal (OP) swab) specimen which collected by a healthcare provider be prepared before test.

The sample collection, storage and handling must be followed CDC guidelines.

(https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html)

- Specimen collection

  : Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing viral transport media.

- Storage

  : Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

- Shipping

  : For domestic and international shipments, specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation External Icon. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

Workflow

Nucleic acids are extracted from upper respiratory (nasopharyngeal (NP) and oropharyngeal (OP) swab) specimens with KingFisher™ Flex Purification system which is an automation extract machine. The Extracted RNA nucleic acid is directly amplified using the A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit on either the QuantStudio™5 Real-time PCR system or CFX96™ Real-time PCR detection system.

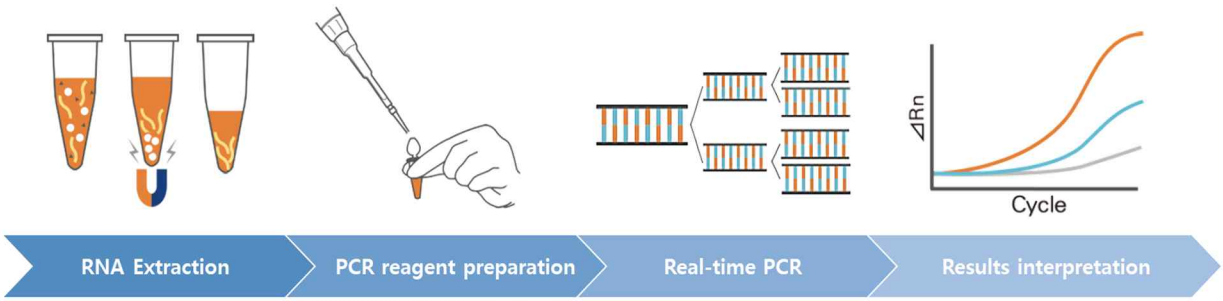


Figure 2. Schematic workflow

1. RNA Extraction

The following extraction device and materials was validated with A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit.

Table 3. Nucleic acid extraction device and materials

	Item	Source
Device	KingFisher™ Flex Purification System	Thermo Fisher Scientific Inc. (Cat. no. N07669)
Reagent	MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher Scientific Inc.
Consumables	KingFisher Flex 96 tip comb	Thermo Fisher Scientific Inc. (Cat. no. 97002534)
	KingFisher 96 KF plate (200 µl)	Thermo Fisher Scientific Inc. (Cat. no. 97002540)
	KingFisher 96 Deep-Well Plates	Thermo Fisher Scientific Inc. (Cat. no. 95040450)

*Extraction device and materials is not included in A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit.

The minimum specimen volume needed for extraction processing is 200㎕ and 50㎕ elution buffer. Following all procedures recommended by the manufacturer of the sample preparation method. Extracted RNA is recommended immediately tested with A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit. If impossible, it should be stored at under -70℃.

2. PCR reagent preparation

※ Thawed all component of this kit thoroughly, mix and centrifuge briefly before use.

※ Please keep the reagent on ice or ice block while using reagent.

※ Before mixing reagents, carefully identify each reagent name.

※ Please vortex

1) Prepared the reagent mixture followed by the table below.

Table 4. Preparation of reagent mixture

Components	Volume per sample
4X master mix	5 ㎕
Oligo mix (ORF1ab)	3 ㎕
Oligo mix (N)	3 ㎕
Oligo mix (Internal control)	4 ㎕
total	15 ㎕

If the test samples is more than 1, please preparing the mixture n (number of reactions) times each component volume and 1 extra sample to allow for sufficient coverage for test. After making reagent mixture. Aliquot 15㎕ in to real-time PCR tubes or plates.

Components	Volume x n
4X master mix	(5 ㎕ x n)+1
Oligo mix (ORF1ab)	(3 ㎕ x n)+1
Oligo mix (N)	(3 ㎕ x n)+1
Oligo mix (Internal control)	(4 ㎕ x n)+1
total	15 ㎕ x n

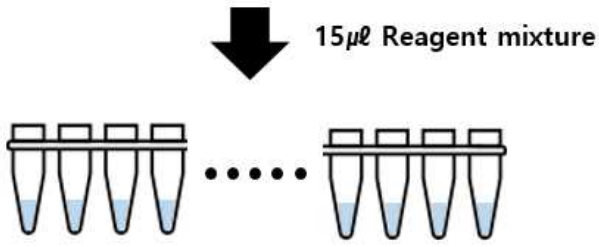


Figure 3. Schematic PCR reagent preparation

2) Add 5㎕ of extracted RNA in 15㎕ of reagent mixture and vortex briefly.

* 1 positive control and 1 negative control are required per machine running. Add 5㎕ each controls.

3) Before run, Plate or tubes are centrifuge briefly.

3. Real-Time PCR set-up (Running)

Set up the PCR conditions on QuantStudio™5 Real-Time PCR System and CFX96 Touch™ Real-Time Detection system instrument as follow.

- **QuantSudio™ 5 Real-Time PCR System**

1) Turn on the power of the computer and turn on the power of the equipment when the window main screen appears.

2) After the equipment is turned on, click "QuantStudio® Design & Analysis Software" to experiment program setting.

3) Set the "Fast Mode" in "Run Mode" of "Properties" Menu.

4) Set the Volume to 20㎕ and PCR stage in "Method" Menu as shown in table 5.

Table 5. PCR stage for Quantstudio™5 Real-Time PCR system

Step	Temperature	Time	Cycle
1	50℃	2 minute	1
2	95℃	20 seconds	1
3	95℃	1 second	40cycles
4*	60℃	15 seconds	

* Fluorescence detection in step 4

5) Set the fluorescence and sample location in "Plate" Menu.
(Set the "N" in the "task" at negative control sample)

Table 6. Fluorescence information

Name	Reporter	Quencher
ORF1ab	FAM	NFQ-MGB
N	HEX (VIC)	NFQ-MGB
Internal control (IC)	Cy5	NFQ-MGB

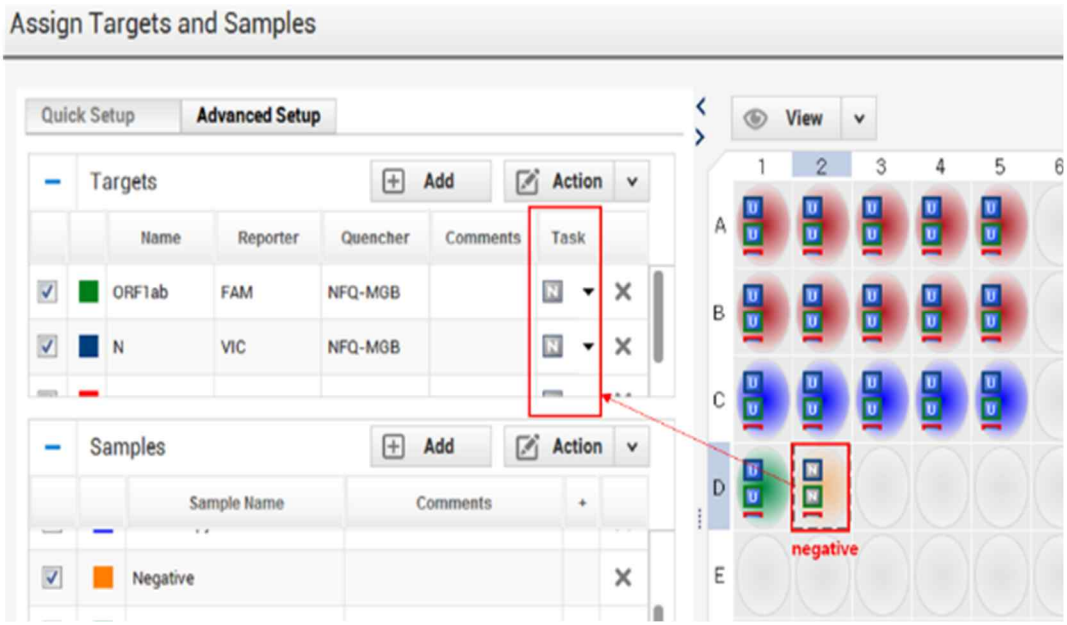


Figure 4. Plate set up

6) Click the start run in "Run" Menu.

- **CFX96 touch™ Real-Time PCR Detection system**

1) Turn on the power of the computer and turn on the power of the equipment when the window main screen appears.

2) After the equipment is turned on, click "Bio-Rad CFX Manager™ Software v3.1"".

3) Click "Create New Experiment" on the screen and "CFX96", which is used equipment.

4) In "Edit selected", set the volume to 20㎕ and set the PCR condition as show in table 7.

Table 7. PCR stage for CFX96 touch™ Real-Time PCR Detection system

Step	Temperature	Time	Cycle
1	50℃	2 minute	1
2	95℃	20 seconds	1
3	95℃	1 second	GOTO 3, 39cycles
4*	60℃	15 seconds	

5) In "Plate", click "Create new" → Click "Select Fluorophores" to select the fluorescent channel (FAM, HEX(VIC) and Cy5) → Click the well containing the sample to check the sample type and fluorescence to be measured. → Click "OK" to save the file.

6) Click "Start Run on Selected Block" in "Start Run" to select the equipment you want to run and click "Start Run".

4. Analyzing the Run Data

Set the thresholds according to the table 8 below after finished PCR instrument.

Table 8. Baseline and Threshold setting on the Real-Time PCR Instruments

Instrument	**QuantStudio™5 Real-Time PCR system (Thermo Fisher)**		
fluorescence	FAM (ORF1ab)	HEX/VIC (N)	Cy5 (IC)
baseline	Auto baseline		
Threshold	Auto baseline		

Instrument	**CFX96 touch™ Real-Time PCR Detection system (Bio-Rad)**					
fluorescence	FAM (ORF1ab)		HEX/VIC (N)		Cy5 (IC)	
baseline	begin	end	begin	end	begin	end
	5	15	10	15	5	15
Threshold	300		80		400	

Result Interpretation

1. Positive and Negative controls

   Please check the Ct value of the positive and negative control as show in table 9. If the Ct value of the controls are not valid, re-testing of all samples from RT-qPCR.

Table 9. The Ct value range of the positive and negative control in A⁺CheQ COVID-19 High-Speed RT-qPCR Detection Kit.

	Positive control	Negative control
ORF1ab gene	21 ± 3	N/D
N gene	26 ± 3	N/D
Internal control	22 ± 3	N/D
Results	Positive	Negative

2. Cut-off Value of the test

   Assessment of clinical specimen test results should be performed after the positive control and the negative control have been determined to be valid and acceptable. If the controls are valid, the patient results can be interpreted.

Table 10. The Cut-off value of A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit.

Target	Ct Value	Result
ORF1ab gene	≤36	positive
	>36, N/A	negative
N gene	≤39	positive
	>39, N/A	negative
Internal control	≤35	positive
	>35	negative

3. Examination and interpretation of the test

Table 11. Data interpretation of the specimens

Case	ORF1ab gene	N gene	Internal control	Result
1	+	+	±	COVID-19 Positive
2	+	-	±	COVID-19 Positive
3	-	+	±	COVID-19 Positive
4	-	-	±	COVID-19 negative
5*	-	-	N/A	Invalid result

* Case5: If the results came out no Ct value, this test is not valid.

※ Please follow by criteria in this document (table8 & table 10) when analyze the results.

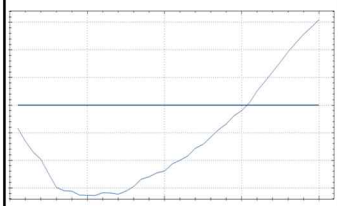
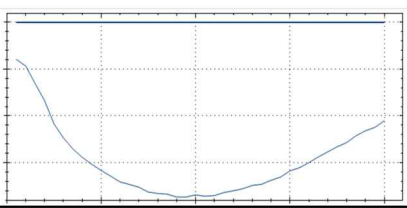
※ Upper interpretation is based on the positive and negative controls data were valid. If the positive and negative control data were in-valid, please all specimens should be retested.

※ In case of cut-off negative judgment threshold, the results come out over cut-off value (36 < ORF1ab(FAM) ≤ 40, 39 < N(HEX) ≤ 40), please re-test with same sample or newly extract from the specimen. If re-test result is same as previous test, please collect the specimen again or do the sequencing the sample for verifying real positive and negative results.

※ In the case of Invalid result, retesting or taking a new sample to test is recommend.

※ Due to false positive and false negative results may occur if virus titer is very low in the specimen, the result should be analyzed by specialist. If necessary, retesting is performed with a residual specimen or newly collection.

Trouble Shooting

Problem	Possible Causes	Solution
No or weak PCR product fluorescent	Using inappropriate consumerble	Use the PCR tube and strip applicable with each machine. Recommend the consumable producted by PCR machine manufacturer.
	insufficient spin-down of plate or strip-tube	After aliquot of reagent and template, PCR plate and strip-tube should go ghrough spin down process thoroughly.
	Wrong Storage condition	Check storage temperature for the reagents and obtain new reagents if needed.
	Expired shelf life	Check the expiration date and use new reagents if needed
	Low template quality	Check template quality using spectrophotometer
	Inhibitors in Template	Check the processing conditions for the sample. Repeat extraction as appropriate.
	Inappropriate Nucleic acid preparation	Check the concentration of nucleic acid. Re-extract the nucleic acid with current specimen or re-collect the specimen if possible.
	Template degradation	Re-test with re-extract template.
	Reagents stored at room temperature	Do not leave reagents at room temperature.
	Unassigned Fluorophore in sample well	Assign the correct fluorophore following the IFU and reanalyze the data.
Non-specific PCR Amplification	Contamination of PCR mixture	Check to confirm that the laboratory environment and equipment have not been contaminated. Clean as needed.
	Contamination from the extraction process	If environment and equipment have not been contaminated, replace RNA extraction reagents.
	insufficient spin-down of PCR tube or plate	After aliquot of reagent and template, PCR plate and strip-tube should be spin down thoroughly.
	abnormal PCR amplification graph	Re-analyse the data with set auto base-line or re test the sample.
False positive / PCR product with non-template control(NTC)	Cross-contamination	Use filter tips, screw-cap tubes and latex gloves. Perform assay set-up in a hood in a clean environment.
Conflicting or unexpected results for different optical channels	Pipette volume error	Check the Pipettes and calibrate as needed.
	Cross contamination	Be careful when you add samples to the PCR tubes.
	If there are foreign objects on the PCR tubes or caps	Remove any debris with a soft cloth before performing the PCR.
No PCR product with positive control or false negative	Template degradation	Do no repeatedly freeze-thaw the positive control (plasmid DNA).
	Incorrect storage	Check storage condition for kit and use a new kit if needed.
	Inappropriate Nucleic acid preparation	Check the concentration of nucleic acid. Re-extract a fresh aliquot of the sample if needed.
	Incorrect PCR mixture (primer & premix) volume	Check the volumes used for the mixture in case of pipetting error.
	Storage of the reagents at room temperature	Do not store the reagents at room temperature and obtain new reagents if needed.
	Using inappropriate consumerble	Use the PCR tube and strip applicable with each machine. Recommend the consumable producted by PCR machine manufacturer.
	insufficient spin-down of plate or strip-tube	After aliquot of reagent and template, PCR plate and strip-tube should be spin down thoroughly.
abnormal PCR amplification graph	Using inappropriate consumerble	Use the PCR tube and strip applicable with each machine. Recommend the consumable producted by PCR machine manufacturer.
	insufficient spin-down of plate or strip-tube	After aliquot of reagent and template, PCR plate and strip-tube should go ghrough spin down process thoroughly.
		Re-analyse the data with set auto base-line or re test the sample. 

Limitation

- A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit is distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.
- 'The test has been validated but FDA's independent review of this validation is pending' should be included in test reports to healthcare providers
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Specimens must be collected, transported, and stored using appropriate procedures and conditions.
- Use of this A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit is limited to personnel who are trained in the procedure. Failure to follow these instructions may lead to erroneous results.
- False negative results may arise from improper specimen collection, handling, and degradation of the viral RNA during shipping/storage.
- False Positive results may arise from the contamination during specimen handling or preparation, or between patient samples.
- Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.
- SARS-CoV-2 may mutate in the target regions of the A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit. If this occurs, then SARS-CoV-2 may not be detected.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not predude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined.

Performance Characteristics

● Analytic Sensitivity of Limit of Detection

To determine the Limit of Detection (LoD) of the A+CheQ COVID-19 High-Speed RT-qPCR Detection kit, studies were performed using SARS-CoV-2 viral RNA which purchased from the National Culture Collection for Pathogen (NCCP, KCDC) in Republic of Korea. SARS-CoV-2 RNA was serial diluted from 10^8 copies/㎕ to 10 copies/㎕. The Detection of Limit (LoD) study established the lowest concentration of SARS-CoV-2 that can be detected by the Kit at least 95% of the time with QuantStudio™5 Real-Time PCR System and CFX96 touch™ Real-Time PCR Detection System instruments.

The LoD of A+CheQ COVID-19 High-Speed RT-qPCR Detection kit was confirmed to be 100 copies/㎕ both QuantStudio™5 Real-Time PCR System and CFX96 touch™ Real-Time PCR Detection System instruments.

Table 12. Limite of Detection (LoD) of A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit

Testing concentration (Copies/㎕)	Replicate	QuantStudio™5 Real-Time PCR system			CFX96 Real-Time PCR system		
		N gene	ORF1ab gene	RNase P gene	N gene	ORF1ab gene	RNase P gene
		Ct Value	Ct Value	Ct Value	Ct Value	Ct Value	Ct Value
100	20	36.80	32.37	24.72	38.12	34.70	28.19
Positive / total		20 / 20	20 / 20	20 / 20	20 / 20	20 / 20	20 / 20
Detection rate , %		100%	100%	100%	100%	100%	100%

● Analytic Sensitivity of Inclusivity

The inclusivity of A+CheQ COVID-19 High-Speed RT-qPCR Detection kit was evaluated using *in silico* analysis by comparison of the primer and probe sequences with 19,267 SARS-CoV-2 sequences published in NCBI database as of July 22, 2020. The ORF1ab gene and N gene primer/probe for SARS-CoV-2 of A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit shows 100% match all of the SARS-CoV-2 sequences.

● Specificity (Cross-Reactivity)

In-silico Analysis

In-silico analysis for the ORF1ab and N primer/probe set of SARS-CoV-2 was conducted to assess cross-reactivity with 29 kinds of organisms.

Table 13. In-silico analysis of cross-reactivity of A⁺CheQ COVID-19 High-Speed RT-qPCR Detection kit

Pathogen	Genbank Accesson	% Homolog					
		ORF1ab Fwd	ORF1ab Rev	ORF1ab Probe	N Fwd	N Rev	N Probe
SARS-CoV-2	NC045512	100%	100%	100%	100%	100%	100%
Bat coronavirus	KY938558	86%	68%	93%	90%	100%	82%
SARS coronavirus	NC004718	90%	74%	96%	90%	100%	88%
MERS coronavirus	NC019843	62%	32%	57%	60%	55%	59%
Human coronavirus 229E	NC002645	62%	32%	54%	25%	23%	29%
Human Coronavirus NL63	NC005831	48%	42%	54%	25%	14%	6%
Human coronavirus OC43	MN310478	52%	26%	46%	30%	14%	24%
Human coronavirus HKU1	KT779555	48%	37%	61%	35%	14%	12%
Influenza A virus (H1N1)	MH798556	0%	0%	0%	0%	0%	0%
Influenza A virus (H3N2)	FJ912901	0%	0%	0%	0%	0%	0%
Influenza B virus	NC002204	0%	0%	0%	0%	0%	0%
Influenza C virus	NC006307.2	0%	0%	0%	0%	0%	0%
Respiratory syncytial virus	NC001803	24%	16%	36%	0%	0%	0%
Human adenovirus	AC000017	14%	26%	29%	25%	23%	35%
Streptococcus pneumoniae	NZLN831051	57%	58%	39%	35%	50%	29%
Salmonella enterica sub sp.	NC003197	62%	47%	46%	45%	41%	65%
Human metapneumovirus	KJ627437	14%	21%	39%	0%	0%	0%
Human enterovirus	NC038308	0%	0%	0%	0%	0%	0%
Human rhinovirus 1	NC038311	0%	0%	0%	0%	0%	0%
Chlamydia pneumoniae	NC005043	52%	37%	54%	40%	59%	47%
Haemophilus influenzae	NZ_LN831035	24%	47%	58%	40%	45%	35%
Legionella pneumophila	NZ_LR134380	48%	37%	29%	30%	59%	53%
Mycobacterium tuberculosis	NC000962	48%	47%	46%	35%	32%	53%
Streptococcus pneumoniae	NZ_LN831051	57%	58%	39%	35%	50%	29%
Streptococcus pyogenes	NZ_LN831034	43%	47%	50%	35%	45%	41%
Bordetella pertussis	NC018518	0%	0%	0%	45%	45%	35%
Mycoplasma pneumoniae	NZ_CP010546	33%	37%	32%	40%	50%	71%
Pseudomonas aeruginosa	NC002516	0%	0%	0%	45%	45%	47%
Staphylococcus epidermidis	NC_004461	52%	47%	39%	35%	59%	41%
Streptococcus salivarius	NZ_LR134274	48%	37%	43%	65%	32%	53%

Results of in silico analysis demonstrates that there is significant homology (> 80%) between the SARS-coronavirus and our Kit primer/probes for N and ORF1ab gene. And Bat coronavirus also shows homology with our Kit primer/probes for N and ORF1ab gene. To verified of the cross-reactivity of our Kit primer/probes for N and ORF1ab gene each oligo set were run the primer blast at NCBI as follow "Primer Pair Specificity Checking Parameters" setting: 1) Set the Organism to SARS coronavirus (taxid:694009) or bat coronavirus (taxid:1508220). The results of NCBI primer blast analysis showed no target template existed. And it was confirmed through the wet testing.

Wet testing

To evaluate the analytical specificity of the A⁺CheQ COVID-19 High-Speed RT-qPCR Detection kit with regards to cross-reactivity, 15 kinds of relevant pathogens's nucleic acid were tested with the A⁺CheQ COVID-19 High-Speed RT-qPCR Detection kit.

The test results showed that no other microorganisms (Related pathogens) were detected in the A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit. It was confirmed that SARS-CoV-2 to be detected specifically.

Table 14. Wet test results of A⁺CheQ COVID-19 High-Speed RT-qPCR Detection kit

Microorganisms	test concentration	Results (6 replicate)		
	Copies/reaction	N gene	ORF1ab gene	Internal control gene
SARS-CoV2 RNA	5×10^6	6/6	6/6	0/6
Human Coronavirus NL63	8×10^5	0/6	0/6	0/6
HCoV- OC43	5×10^6	0/6	0/6	0/6
HCoV-HKU1	5×10^6	0/6	0/6	0/6
HCoV- 229E	5×10^6	0/6	0/6	0/6
HCoV- MERS	5×10^6	0/6	0/6	0/6
HCoV- SARS	5×10^6	0/6	0/6	0/6
Influenza A virus (H1N1)	1.8×10^6	0/6	0/6	0/6
Influenza A virus (H3N2)	1.8×10^6	0/6	0/6	0/6
Influenza B virus	1.65×10^6	0/6	0/6	0/6
Respiratory syncytial virus	1.85×10^6	0/6	0/6	0/6
Adenovirus type1	3.15×10^6	0/6	0/6	0/6
Mycobacterium tuberculosis gDNA	1.58×10^6	0/6	0/6	0/6
Streptococcus pneumonia gDNA	1.5×10^6	0/6	0/6	0/6
Salmonella Typhimurium	5×10^6	0/6	0/6	0/6
Human RNA	5×10^6	0/6	0/6	6/6*

**** Internal control designed for Human gene detection.***

● Clinical Evaluation

The clinical evaluation test was performed to evaluate the A⁺CheQ COVID-19 High-Speed RT-qPCR Detection kit using upper respiratory tract (Oropharyngeal swab, Nasopharyngeal swab) specimens.

For this study, A total of 84 (42 positive, 42 negative) specimens were extracted automatically by KingFisher™ Flex Purification System and evaluated with QuantStudio™ 5 Real-time PCR system (Thermofisher). Results of the clinical evaluation test are summarized in table 15.

Table 15. Clinical Evaluation test of A⁺CheQ COVID-19 High-Speed RT-qPCR Detection kit

Nasopharyngeal / Oropharyngeal (throat) swab		**Comparator test**		
		Positive	Negative	total
A+CheQ COVID-19 High-Speed RT-qPCR Detection Kit	Positive	42	0	42
	Negative	0	42	42
	total	42	42	84
Positive Agreement(PPA) : 42/42, 100% (95% CI, 91.62% – 100%) **Negative Agreement(NPA) : 42/42, 100% (95% CI, 91.62% – 100%)**				

Performance was estimated as 100% agreement with positive and negative sample of upper respiratory specimen types.

Technical Support

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Description of Symbols

Symbol	Explanation	Symbol	Explanation
EUA	Emergency Use Authorization	LOT	Batch code
IVD	In-vitro diagnostics Medical devices	**Rx only**	Prescription only use
	Caution		Sufficient for <n> tests
	Temperature limit		Consult instructions for use
	Manufacture date		Use by date
CE	CE mark	EC REP	Authorized representative in the European community
	Manufacturer		Keep away from Sunlight