CE IVD

AQ-TOP™ COVID-19 Rapid Detection Kit

For in vitro diagnostic use only

RX only

For use under Emergency Use Authorization (EUA) only

A molecular rapid diagnostic kit for detection of SARS-CoV-2 (COVID-19) in clinical samples using real-time Isothermal Amplification

Instructions for Use | V1.2

Store at –20°C

Date of Revision: January-2022

Certificate of KGMP

ISO 13485 LL-C (Certification) Certificate of ISO13485

SEASUN BIOMATERIALS

Indications of Medical Devices Act

1. Product Category:	IVD Reagent for Infectious Agents
2. Product Name:	AQ-TOP™ COVID-19 Rapid Detection Kit
3. Product Catalogue Number:	SS-9920
4. Purpose of use:	See 1. in this User Guide

Warnings and Precautions

Contact us for detailed information for the safe use of the AQ-TOP™ COVID-19 Rapid Detection Kit. Please check storage temperature and attention points for accurate diagnosis of the product. Sample and Assay waste must be disposed of in a legally designated manner.

Warranty and Responsibility

All products of SEASUN BIOMATERIALS Inc. are tested under rigorous quality management processes. SEASUN BIOMATERIALS Inc. guarantees to ensure the quality of the product during warranty period. If any problems relating to the quality of the product are found, please contact the headquarters immediately.

Quality Control System

All aspects of the quality management system, product creation, quality assurance, and supplier qualifications are certified to ISO 13485, ISO 9001, KGMP.

Inquiries and customer service (A/S)

Send us an e-mail (as@seasunbio.com) to inquire about the product.

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1. Intended Use

The AQ-TOP™ COVID-19 Rapid Detection Kit is a Real-Time Loop Mediated Isothermal Amplification (RT-LAMP) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens including oropharyngeal and nasopharyngeal swab specimens, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirate specimens, bronchoalveolar lavage (BAL) and sputum from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to 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 upper and lower 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 diagnostics 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. Laboratories within the United States and its territories are required to report all 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 AQ-TOP™ COVID-19 Rapid Detection Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR/LAMP and in vitro diagnostic procedures. The AQ-TOP™ COVID-19 Rapid Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Product Description

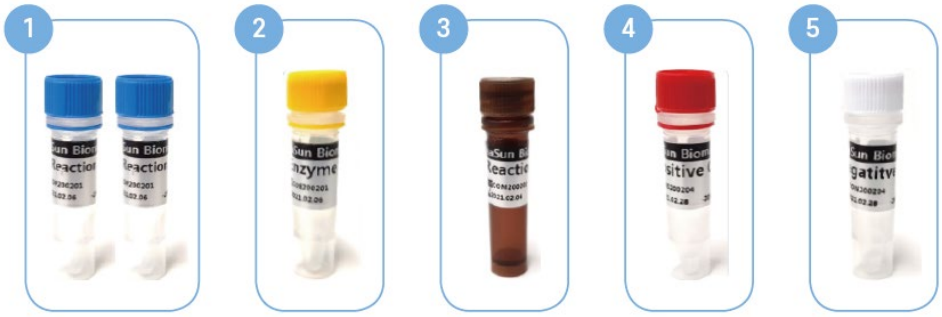
The AQ-TOP™ COVID-19 Rapid Detection Kit is a qualitative test based on RT-LAMP for detection of the SARS-CoV-2 RNA extracted from clinical specimens collected using the Viral Sample Collection Kit (Noble Bio, Cat # UTNFS-3B-1 or sterile sputum collection container (BD, Cat # 90004-118).

The AQ-TOP™ COVID-19 Rapid Detection Kit uses dual-labeled Peptide Nucleic Acid (PNA) probes that target ORF1ab for detection of SARS-CoV-2 RNA in FAM, and human RNase P for the internal control (IC) in the HEX fluorescence channel. Both reverse transcription and LAMP reactions take place at 60°C using the enzyme mixture of Reverse Transcriptase and Bst Polymerase. During the amplification, fluorescence resonance energy transfer (FRET) probes are incorporated in the amplification products. Upon incorporation, fluorescence is generated and can be monitored by the fluorescence reader on the CFX 96 and ABI 7500 real-time PCR platforms in a real time fashion.

In addition, the kit utilizes external Positive (PC) and Negative (NC) controls. The PC contains templates for SARS-CoV-2 Orf1ab and the human RNase P gene. The NC contains RNase/DNase free distilled water.

3. Kit Components and Packaging Specifications

The kit is composed of 2X Reaction buffer, Enzyme Mix, Reaction Mix, Positive control and Negative Control.



	Reagent label	Part #	Descriptions/Contents	Volume / Quantity	Store at
1	2X Reaction buffer	SS-9920CVB	PCR buffer	750㎕ / 2 tubes	-20°C
2	Enzyme Mix	SS-9920CVE	Reverse transcriptase Bst polymerase	100㎕ / 1 tube	
3	Reaction Mix	SS-9920CVM	Primer, probe mixture	400㎕ / 1 tube	
4	Positive Control	SS-9920CVP	Templates for SARS-CoV-2 and RNase P	200㎕ / 1 tube	
5	Negative Control	SS-9920CVN	Nuclease free DW	200㎕ / 1 tube	

Quality Control

Negative Control (NC): contains nuclease-free water intended to evaluate cross contamination of the kit, supplements, reagents and PCR instrument used in the test. Detection accuracy can be evaluated with the NC as well as non-specific signals that may be caused by primer dimer, primer-probe non-specific binding. The Negative control should be run using 10 µL in one well per test.

Positive Control (PC): contains plasmids with insert of SARS-CoV-2 Orf1ab and the human RNase P that is intended to evaluate RNA extraction, Enzyme activity, and Analytical and Clinical performance of the kit. The positive control should be run using 10 µL in one well

per test.

Internal Control (IC): The Reaction Mix tube of the kit consists of a primer set and a probe that detects human RNase P. The internal control is intended to evaluate the RNA extraction process, test accuracy as well as the real-time PCR instrument performance.

Both PC and NC should be used directly with the test without prior dilution.

4. Storage and Handling Requirements

Store all reagents at –20°C (both un-opened and in-use product).

Use the reagents within 3 months once opened.

Reagents should not be used past their expiration date.

Completely thaw the reagents except the Enzyme mix at room temperature before each use.

Place all reagents on ice once thawed during the whole test procedure.

Place Enzyme Mix on ice during the whole test procedure.

Avoid excessive freeze/thaw cycles.

Vortex and spin down briefly the reagents before each use.

5. Additional Materials and Equipment

The kit does not include sample collection and preservation instruments/buffers, RNA extraction reagents and Real-time PCR detection systems. Components required for detection of SARS-CoV-2 but not included with the kit are:

1. Sample collection / Storage / Shipping consumables
   A. Viral Sample Collection Kit (Noble Bio, Cat.No UTNFS-3B-1) for collection and transport of upper respiratory tract specimens.
   B. Sputum collection container (BD, Cat # 90004-118) for collection and transport of lower respiratory tract specimens (including BALs).
2. RNA extraction kit for extracting RNA from clinical specimens.
   A. QIAamp DSP virus kit (Qiagen, Cat. No 60704)
3. Real-time PCR system and the consumables
   A. Real-time PCR detection systems can be used with the AQ-TOP™ COVID-19 assay are
      -CFX 96 real-time PCR detection system (Bio-Rad) with software CFX manager

V3.1

-Applied Biosystems real-time PCR system 7500 with Software 2.0.6

B. Consumables

- 96 well white PCR plate (Bio-Rad MLL9651, Applied Biosystems AB0900W or equivalent)
- Sealing Film or 8-12 well PCR plate cap (Bio-Rad MSB 1001 or equivalent)
- Vortex and Micro centrifuge
- Sterilized pipette tips with filter (10 μL, 200 μL and 1000 μL)
- 1.5 mL DNase/RNase free microcentrifuge tubes and racks
- Disposable powder-free gloves and laboratory gowns

6. Warnings and Precautions

For *in vitro* diagnostic use only.

This test has not been FDA cleared or approved.

This test has been authorized by FDA under an EUA for use by authorized Laboratories.

This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Use under the guidance of physicians and specialists.

Please read this user guide carefully before first use.

Sensitivity of reagents may be lowered with prolonged exposure to room temperature or light.

Store all assay contents at -20℃ away from UV/sunlight.

Avoid use if the kit is contaminated with test sample.

Keep clear the external environment, always use in a clean place.

Only use sterilized single-use micro filter tips.

Strong external impact may damage the screw tube.

If any abnormality is observed, stop the experiment, contact the manufacturer.

7. Specimen Collection, Handling and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

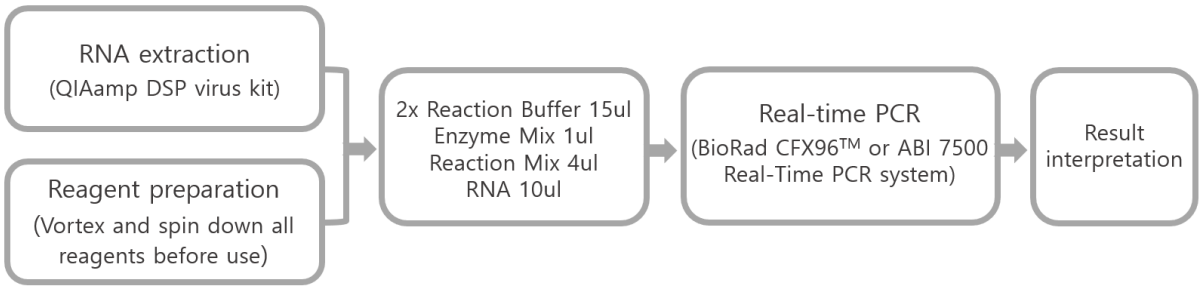
Collecting specimen: Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2)(https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Follow specimen collection device manufacturer instructions for proper collection methods. Swab specimens should be collected using only swabs with a synthetic tip, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 mL of viral transport media. Store the samples at 2-8°C up to 72 hours. If a delay in shipping or extraction is expected, store samples at –70°C.

Shipping: Specimens must be packaged and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Store specimens at 2-8°C and ship to the lab on ice packs. If a specimen is frozen at -70°C, ship to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

Rejection criteria: Specimens will be rejected prior to the test 1) If the specimens were stored at 2-8°C over 72 hours 2) Specimens without sufficient volume for the test (less than 1 mL) 3) Label is damaged (cannot be read or recognized) or without labeling/identifying documents.

8. Test procedure

8.1 Summary of Preparation and Testing Process



Work flow of AQ-TOP™ COVID-19 Rapid Detection Kit

8.2 RNA extraction from clinical specimens

The AQ-TOP™ COVID-19 Rapid Detection Kit does not include viral RNA extraction reagents. The QIAamp DSP virus kit (Qiagen, Cat # 60704) has been validated with the AQ-TOP™ COVID-19 Rapid Detection Kit. The extraction kit requires 300 µL of sample input (both upper and lower respiratory tract specimens) and yields 60 µL of purified nucleic acid eluent. Following the extraction, RNA should be used immediately or stored at -70°C (for up to 1 month) for later use.

8.3 Reaction master mix and Assay set up

Note: Plate set-up configuration can vary with the number of specimens. Negative and Positive control must be included in each run. Prepare reaction master mix in separate area (Assay preparation area) from nucleic acid handling.

1. Clean and decontaminate all work surfaces, equipment as well as small supplements e.g. pipette, vortex, micro centrifuge with 70% ethanol prior to use to minimize the risk of nucleic acid cross-contamination.
2. Place enzyme mix on ice during the whole test procedure. Other reagents can be thawed at room temperature. Keep all reagents on ice once thawed during the whole test procedure.
3. Vortex for 5 sec and spin down all reagents briefly before use.
4. Determine the number of reactions to set up for the assay. Be sure to make excess reaction mix for PC, NC and for possible pipetting error.

5. Prepare the reaction master mix in a 1.5 mL microcentrifuge tube according to the following table. It is recommended to prepare 110% of the calculated amount of master mix to account for pipetting carryovers.

Master mix for one reaction

Reagents	Volume (µL)
2X Reaction Buffer	15
Enzyme Mix	1
Reaction Mix	4
Total (w/o RNA sample)	**20**

6. Vortex the prepared master mix for 5 sec and centrifuge briefly to collect contents at the bottom of the tube and place the tube in a cold rack (ice or cold block).
7. Set up 96 well PCR plate.
8. Dispense 20 µL of master mix into the wells of 96-well PCR plate.
9. Pipette **10 µL** of NC into NC sample well (dispensing sample and control in 96 well plate are irrelevant, no fixed position is required).

Nucleic acid template addition

Note: Always change pipette tips in-between patient sample handling and after pipetting each component. Add the Positive Control to the PCR plate last, to avoid possible contamination. The Positive Control contains a high concentration of viral template. Change gloves often to avoid cross contamination between samples and control reagents

1. Gently vortex clinical RNA extract tubes for approximately 5 sec and spin down to collect contents at the bottom of the tubes. Always keep the sample tubes on ice or in a cold block.
2. Dispense nucleic acid samples of **10 µL** into the 96 well PCR plate containing the aliquoted reaction master mix.
3. Carefully pipette **10 µL** of PC into a PCR plate well last.
4. Seal the PCR plate with cap strip or sealing film. Ensure the sealing film is completely

absorbed to the plate by using a roller.

5. Spin down briefly using a micro plate centrifuge to downward the contents and remove extra air bubbles. It is recommended to centrifuge for 30 sec at 500 x g, 4°C.

8.4 Set up real-time LAMP run

Note: AQ-TOP™ COVID-19 Rapid detection kit running protocol is slightly different for the CFX 96 and ABI 7500 real-time PCR detection systems. The run protocol and fluorescence channels for the targets are shown in Tables 1 and 2.

Table 1. RT-LAMP Conditions

Instrument	Temp	Time	Repeat
CFX96	60℃	50 sec	30*
ABI 7500		60 sec	

* Collect fluorescence signal in each repeat

Table 2. Fluorescence Channel for Probes

Fluorescence	Target
FAM	<Orf1ab> SARS-CoV-2
HEX/VIC or JOE*	<RNase P> human

* HEX for Bio-Rad CFX 96, VIC or JOE for ABI 7500 platform

CFX96 and Software Operation - 1 (New experiment)

① Turn on a computer and CFX 96 > Display the 96-well thermal block > Place the 96 well plate prepared in previous step.

② Run the CFX Manager software on the computer connected to the CFX 96. Go to File > New > Protocol > input the run information as shown in Table 1. > Set the sample volume to 30 µL.

③ Go to Plate > Edit Selected > Set Fluorophores > Select fluorescence channel FAM and HEX.

④ Specify the positive control well, select “Positive Control” from “Sample type”, and load the fluorophores.

⑤ Specify the negative control well, select “Negative Control” from “Sample type”, and

load the fluorophores.

⑥ Wells with clinical specimens should be specified as Unknown, and load the fluorophores.

⑦ Go to Settings > plate type > Select BR white.

⑧ Go to Start Run > Select Block Name (PCR instrument) to use > Close Lid and Start Run.

CFX96 and Software Operation - 2 (Pre-Programmed Run Settings)

① If you have a previous run file, you can re-use the programmed conditions for additional runs. Double click on a previous run file and select sequentially File > Repeat Run.

② Go to Plate tab > set Control and Sample information > Start Run. The fluorescence channel, plate type, and volume are already selected with previous run.

ABI 7500 and Software Operation – 1 (New experiment)

① Turn on a computer and ABI 7500 > display the 96 well thermal block > Place the 96 well plate prepared in previous step.

③ Run 7500 Software on the computer connected to the ABI 7500. Select sequentially 7500 (96 well) > Quantitation-Standard curve > TaqMan@ Reagents > Standard (2 hours to complete a run).

④ Go to Plate Setup > Define Targets and Samples > Define Targets > Add New Target > Set Target Name and Reporter as shown below:

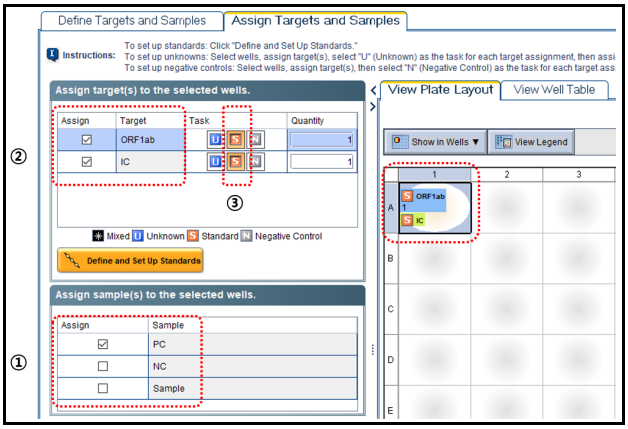
Target 1. ORF1ab: Reporter FAM; Quencher NFQ-MGB

Target 2. IC: Reporter VIC (or JOE); Quencher NFQ-MGB

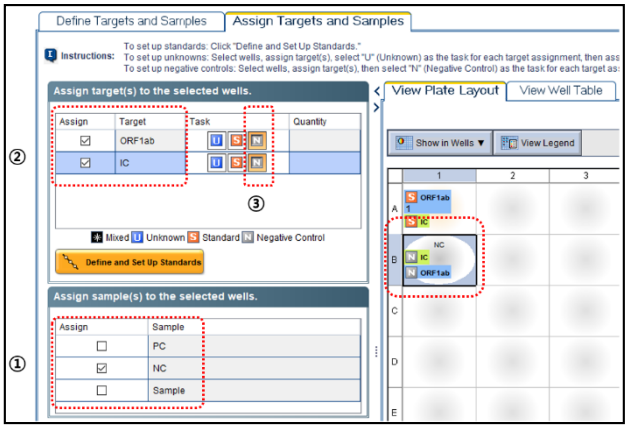
⑤ Go to Define Samples > Add New Sample > Input PC, NC and Sample (Test Specimen).

⑥ Go to "Assign Target and Samples" to set targets and well positions for PC, NC and Samples to be analyzed.

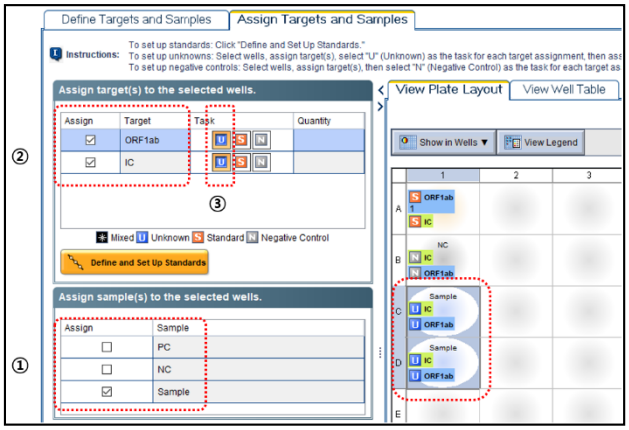
1. Positive Control: Click Positive Control Well from "View Plate Layout". Select "PC" from ① (shown in figure below) and activate both targets from ② (shown in figure below) then activate "**S**" for both targets from ③ (shown in figure below).



2. Negative Control: Click Negative Control Well from "View Plate Layout". Select "NC" from ① (shown in figure below) and activate both targets from ② (shown in figure below) then activate "**N**" for both targets from ③ (shown in figure below)



3. Sample: Click Wells with test samples from "View Plate Layout". Select "Sample "from ① (shown in figure below) and activate both targets from ② (shown in figure below).



⑦ Select “None” from “Select the dye to use as the passive reference.”

⑧ Go to Run Method > Input the PCR condition as shown in Table 1. Setting with Tabular View is easier than with Graphical View. Set “Reaction volume Per Well” to 30 μL.

⑨ Save the protocol from File > Save As, then Go to Run and click “START RUN“ to start amplification.

ABI7500 and Software Operation - 2 (Pre-Programmed Run Settings)

① A previous run file can be used as a template. Go to File > Open > Select the file.

② Input the sample information in the “Plate setup” and proceed in the same order as above.

9. Result Interpretation

Base line and threshold setting

The AQ-TOP™ COVID-19 Rapid Detection Kit has been validated using the baseline threshold setting which is automatically adjusted by both the CFX 96 and ABI 7500 Real-Time PCR instruments.

Interpretation of Quality control

All controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Negative Control: The NC reaction for fluorescence channels should not exhibit any fluorescence growth curves (Ct) that cross the threshold line ≤ 30.

Positive Control: The PC will yield a positive result with both FAM and HEX or VIC/JOE fluorescence channels (FAM for SARS-CoV-2 Orf1ab, HEX or VIC/JOE for the human RNase P).

If control results are invalid, check condition of kit storage, reaction condition and master mix preparation step. Repeat the run using the same reagents with strict adherence to the guidelines. If controls exhibit invalid results again discard the control tubes and re-test using new controls. If the invalid result is generated for a third time with new control reagents, discard the whole kit.

The controls should meet the requirements listed in Table 3 to ensure valid results.

Table 3. Interpretation of Results for Quality Control

Control	Ct value	
	ORF1ab (FAM)	IC (HEX or VIC/JOE)
Negative	ND	ND
Positive	≤ 30	≤ 30

ND= Not detectable

Interpretation of Clinical samples

If the values of the controls are conclusive, refer to the table (Table 4) below to determine the infection status of the patient sample.

Table 4. Clinical Sample Results Interpretation

Ct value		Interpretation	Action
ORF1ab (FAM)	**IC (HEX or VIC/JOE)**		
> 30 or ND	≤ 30	Negative (Absence of SARS-CoV-2 RNA)	Report results to healthcare provider. Consider testing for other viruses that may cause similar symptoms.
≤ 30	/	Positive (Presence of SARS-CoV-2 RNA)	Report results to healthcare provider and appropriate public health authorities.
> 30 or ND	> 30 or ND	Invalid	Repeat test with same RNA extract if available. If result remains invalid, repeat the extraction procedure with the remaining clinical specimen and repeat the test. If all markers remain negative after re-test, report the results as invalid and re-collect patient sample.

/ = No requirement of Ct value: If the SARS-CoV-2 target (ORF1ab) has a Ct value of ≤ 30, the Ct value of the IC is not required to be considered. It is possible, that samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample.

Internal Control (IC): Failure to detect RNase P in any clinical specimen may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
- Improper assay set up and execution.
- Reagent or equipment malfunction.

10. Limitations

- This test is a qualitative test and does not provide the quantitative value of viral load in the original specimens.
- The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
- Extraction of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches have not been evaluated.
- Amplification and detection of SARS-CoV-2 with this kit has only been validated with the CFX-96 Real-time PCR Detection system and Applied Biosystems® 7500 Real-Time PCR instrument. Use of other instrument systems may cause inaccurate results.
- False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

11. Conditions of Authorization for the Laboratory

The AQ-TOP™ COVID-19 Rapid Detection Kit assay's Letter of Authorization, User Manual, and Labeling are available on FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

To assist clinical laboratories using the AQ-TOP™ COVID-19 Rapid Detection Kit, the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

b) Authorized laboratories using your product will use your product as outlined in the Instructions for Use only. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

c) Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

d) Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities.

e) Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and SEASUN BIOMATERIALS (via email: info@seasunbio.com) if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product.

f) All laboratory personnel using your product must be appropriately trained in molecular techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

g) SEASUN BIOMATERIALS, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

12. Assay Performance

12.1 Limit of Detection (LoD)

The LoD study established the lowest SARS-CoV-2 viral RNA concentration (genomic copies/ul) that consistently yielded a 95% positivity rate with the AQ-TOP™ COVID-19 Rapid Detection Kit.

A preliminary LoD for the SARS-CoV-2 specific target ORF1ab was determined using whole viral genomic RNA (NCCP No. 43326. National Culture Collection for Pathogens) spiked into pooled negative clinical nasopharyngeal swab and sputum matrices. In the first part of this study, 10-fold dilutions of known concentrations of genomic RNA were prepared in negative clinical matrix (both nasopharyngeal swab and sputum) and processed using the Qiagen QIAamp DSP virus kit and run on the CFX 96-real time PCR detection system. Five PCR replicates per concentration were tested. See Table 5 for a summary of the LoD range finding study:

Table 5. Summary of Preliminary LoD testing

A. Sputum specimen

	70,000 copies/µL		7,000 copies/µL		700 copies/µL		70 copies/µL		7 copies/µL	
	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P
+/total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Mean Ct	10.32	23.22	11.47	23.73	13.46	23.47	15.24	23.33	18.26	24.43
SD	0.13	0.53	0.35	0.87	0.39	0.61	0.55	0.61	1.00	0.55

B. Nasopharyngeal swab specimen

	70,000 copies/µL		7,000 copies/µL		700 copies/µL		70 copies/µL		7 copies/µL	
	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P
+/total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Mean Ct	9.99	23.77	11.65	23.22	13.19	23.53	15.16	23.77	18.41	23.85
SD	0.30	0.59	0.51	0.53	0.36	0.74	0.57	0.85	1.09	0.72

Based on these results, additional 3-fold dilutions of known concentrations of genomic RNA were prepared in negative clinical matrix (nasopharyngeal swab and sputum). Twenty (20)

individual extraction replicates per dilution were tested on both the CFX 96 and ABI 7500 real-time PCR systems. The lowest target level at which more than 95% of 20 replicates produced positive results was 7 copies/ μL for both PCR platforms (CFX 96 and ABI 7500 system) for both upper and lower tract specimens (Table 6).

Table 6. Summary of LoD confirmation

A. Sputum specimen

	CFX 96 real-time PCR system						**ABI 7500 real-time PCR system**					
	7 copies/μL		2.3 copies/μL		0.8 copies/μL		7 copies/μL		2.3 copies/μL		0.8 copies/μL	
	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC
+/total	20/20	20/20	14/20	20/20	8/20	20/20	20/20	20/20	11/20	20/20	9/20	20/20
Mean Ct	18.77	23.16	-	23.72	-	22.93	18.53	23.23	-	23.72	-	23.39
SD	0.65	0.45	-	0.73	-	0.39	0.64	1.02	-	0.73	-	0.94

B. Nasopharyngeal swab specimen

	CFX 96 real-time PCR system						**ABI 7500 real-time PCR system**					
	7 copies/μL		2.3 copies/μL		0.8 copies/μL		7 copies/μL		2.3 copies/μL		0.8 copies/μL	
	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC	Orf1ab	IC
+/total	20/20	20/20	15/20	20/20	11/20	20/20	20/20	20/20	12/20	20/20	8/20	20/20
Mean Ct	18.73	23.17	-	23.64	-	22.92	18.77	23.16	-	23.72	-	22.93
SD	0.58	0.44	-	0.70	-	0.39	0.65	0.45	-	0.73	-	0.39

12.2 Inclusivity (Analytical Reactivity)

Analytical reactivity (inclusivity) of the AQ-TOP™ COVID-19 Rapid Detection Kit was evaluated using publicly available full and partial SARS-CoV-2 genome sequences. 5876 sequences were downloaded from the following databases including National Genomics Data Center China (https://bigd.big.ac.cn/), GenBank (https://www.ncbi.nlm.nih.gov/genbank/), GISAID (https://www.gisaid.org/), GWH (https://bigd.big.ac.cn/gwh/) and NMDC (https://microbiomedata.org/).

Analysis was performed using the <Find binding sites and create fragment> tool in CLC main workbench 20.0.3 software. 496 sequences which comprise whole genome information of SARS-CoV-2 were analyzed against the primer and probes contained in the kit. All the alignments of the kit's primer and probe sets against the available 496 SARS-CoV-2 sequences showed 100% identity (absence of mismatch base against the SARS-CoV-2 target).

12.3 Specificity (Cross-Reactivity)

Evaluation of analytical specificity of the kit was conducted using both in-silico analysis and wet testing against pathogenic organisms mainly found in the human respiratory tract.

In-silico Analysis:

BLASTn analysis queries of the AQ-TOP™ COVID-19 Rapid Detection Kit primers and probes were performed against public domain nucleotide sequences with the following database search parameters:

- Mask low complexity regions = Yes
- Expectation value = 10
- Match/Mismatch = Match 2 Mismatch -3
- Gap Costs = Existence 5 Extension 2
- Max number of hit sequence = 250
- Mask lower case = No
- Mask low complexity regions = Yes
- Number of threads = 16
- Filter out redundant results = No.

However, some of the primers showed high homologies to specific microorganisms: SARS-coronavirus, *Haemophilus influenzae, Legionella pneumophila, Streptococcus pneumoniae, Streptococcus pyogenes, Mycoplasma pneumoniae, Staphylococcus epidermis, Streptococcus salivarius* and *Staphylococcus aureus.* Since the amplification and detection of RT-LAMP requires simultaneous binding of six (6) primers and a detection probe to the target nucleic acid, it is not expected that these microorganisms will be amplified or produce cross-reactive signal. Because simultaneous homologies were only exhibited to 4 primers at most. Result of the in-silico analysis is shown in table 7.

Table 7. In-silico Cross-Reactivity Analysis

Microorganism	Reference No	Primer						Detection Probe
		F3_4	B3_4	FIP_4	BIP_4	LoopF4	LoopB4	
Human coronavirus 229E	NC_002645.1	/	/	/	/	/	/	/
Human coronavirus OC43	NC_006213.1	/	/	/	/	/	/	/
Human coronavirus HKU1	NC_006577	/	/	/	/	/	/	/
Human coronavirus NL63	NC_005831.2	/	/	/	/	/	/	/
SARS-coronavirus	NC_004718.3	100%	/	/	86%	90%	100%	/
MERS-coronavirus	KJ556336.1	/	/	/	/	/	/	/
Adenovirus type 1	MH183293.1	/	/	/	/	/	/	/
Adenovirus type 2	J01917.1	/	/	/	/	/	/	/
Adenovirus type 3	AY599836.1	/	/	/	/	/	/	/
Human Metapneumovirus	KJ627437.1	/	/	/	/	/	/	/
Parainfluenza virus 1	KX639498.1	/	/	/	/	/	/	/
Parainfluenza virus 2	KM190939.1	/	/	/	/	/	/	/
Parainfluenza virus 3	NC_001796.2	/	/	/	/	/	/	/
Parainfluenza virus 4	JQ241176.1	/	/	/	/	/	/	/
Influenza A	GCF_000865085.1	/	/	/	/	/	/	/
Influenza B	BLee1940	/	/	/	/	/	/	/
Enterovirus	NC_001472.1	/	/	/	/	/	/	/
Respiratory syncytial virus	NC_001803.1	/	/	/	/	/	/	/
Rhinovirus	NC_009996.1	/	/	/	/	/	/	/
Chlamydia pneumoniae	NC_005043.1	/	/	/	/	/	/	/
Haemophilus influenzae	NZ_LN831035.1	/	/	68%	/	/	/	/
Legionella pneumophila	NZ_LR134380.1	78%	/	/	/	/	/	/
Mycobacterium tuberculosis	NC_000962.3	/	/	/	/	/	64%	/
Streptococcus pneumoniae	NZ_LN831051.1	61%	/	/	/	/	/	/

Streptococcus pyogenes	NZ_LN831034.1	/	/	/	/	94%	/	80%
Bordetella pertussis	NC_018518.1	/	/	/	/	/	/	/
Mycoplasma pneumoniae	NZ_CP010546.1	/	/	/	/	/	76%	/
Pneumocystis jirovecii	CAKM01000281.1	/	/	/	/	/	/	/
Candida albicans	GCA_003454745.1	/	/	/	/	/	/	/
Pseudomonas aeruginosa	NC_002516.2	/	/	/	/	/	/	/
Staphylococcus epidermidis	NZ_CP035288.1	/	78%	73%	/	70%	82%	/
Streptococcus salivarius	GCF_900636435.1	/	/	63%	/	/	64%	/
Staphylococcus aureus	BX571856.1	/	89%	/	/	/	76%	/

/ = no alignment found

Cross-Reactivity Wet Testing

Wet testing against normal and pathogenic organisms of the respiratory tract was performed to confirm the results of the in-silico analysis. Each organism (cultured isolates or inactivated strains) identified in Table 8 was tested using three extraction replicates with the AQ-TOP™ COVID-19 Rapid Detection Kit at concentrations of 10^6 CFU/mL or higher for bacteria and 10^5 pfu/mL or higher for viruses. No detectable amplification curve (Ct) was observed in the FAM detection channel for the SARS-CoV-2 ORF1ab when using the CFX 96 platform. As expected, the internal control did show 100% detection for all three tested replicates for all organisms evaluated for potential cross-reactivity.

Table 8. Cross-Reactivity Wet Testing Analysis

Microorganism	Source	% Detection (#detected / #tested)	
		ORF1ab	IC
Human coronavirus 229E	KBPV[a] VR-9	0% (0/3)	100% (3/3)
Human coronavirus OC43	KBPV VR-8	0% (0/3)	100% (3/3)
Human coronavirus HKU1	ATCCVR-3262SD[b]	0% (0/3)	100% (3/3)
Human coronavirus NL63	NCCP 43214	0% (0/3)	100% (3/3)
SARS-coronavirus	Clinical isolate[c]	0% (0/3)	100% (3/3)
MERS-coronavirus	Clinical isolate	0% (0/3)	100% (3/3)
Adenovirus type 1	KBPV VR-1	0% (0/3)	100% (3/3)
Adenovirus type 2	KBPV VR-58	0% (0/3)	100% (3/3)
Adenovirus type 3	KBPV VR-2	0% (0/3)	100% (3/3)
Human Metapneumovirus	KBPV VR-86	0% (0/3)	100% (3/3)
Parainfluenza virus 1	KBPV VR-44	0% (0/3)	100% (3/3)
Parainfluenza virus 2	KBPV VR-45	0% (0/3)	100% (3/3)
Parainfluenza virus 3	KBPV VR-46	0% (0/3)	100% (3/3)
Parainfluenza virus 4	KBPV VR-69	0% (0/3)	100% (3/3)
Influenza A (H3N2)	KBPV VR-32	0% (0/3)	100% (3/3)
Influenza A (H1N1)	KBPV VR-33	0% (0/3)	100% (3/3)
Influenza B	KBPV VR-34	0% (0/3)	100% (3/3)

Enterovirus	KBPV VR-12	0% (0/3)	100% (3/3)
Respiratory syncytial virus	KBPV VR-48	0% (0/3)	100% (3/3)
Rhinovirus 1	KBPV VR-1	0% (0/3)	100% (3/3)
Rhinovirus 14	KBPV VR-39	0% (0/3)	100% (3/3)
Rhinovirus 7	KBPV VR-82	0% (0/3)	100% (3/3)
Chlamydia pneumoniae	ATCC 53592	0% (0/3)	100% (3/3)
Haemophilus influenzae	CCARM 9257	0% (0/3)	100% (3/3)
Legionella pneumophila	CCARM 19001	0% (0/3)	100% (3/3)
Mycobacterium tuberculosis	NCCP15972	0% (0/3)	100% (3/3)
Streptococcus pneumoniae	CCARM 4157	0% (0/3)	100% (3/3)
Streptococcus pyogenes	CCARM 4528	0% (0/3)	100% (3/3)
Bordetella pertussis	NCCP 13671	0% (0/3)	100% (3/3)
Mycoplasma pneumoniae	ATCC 29342	0% (0/3)	100% (3/3)
Pneumocystis jirovecii (PJP)	Lab culture[c]	0% (0/3)	100% (3/3)
Candida albicans	CCARM 14004	0% (0/3)	100% (3/3)
Pseudomonas aeruginosa	CCARM 0220	0% (0/3)	100% (3/3)
Staphylococcus epidermidis	CCARM 3711	0% (0/3)	100% (3/3)
Streptococcus salivarius	NCCP 14735	0% (0/3)	100% (3/3)
Staphylococcus aureus	NCCP 15920	0% (0/3)	100% (3/3)
Nasal wash	-	0% (0/3)	100% (3/3)

[a]; KBPV: Korean bank of pathogenic virus (https://www.kbpv.re.kr/index.php)
[b]; human coronavirus HKU1 was tested using spiked isolated nucleic acid at a concentration of $5x10^5$ copies/mL
[c]; Clinical isolate, Culture: Clinical isolates in department of diagnostics, Hospital of Chungnam University, Korea.

12.4 Clinical Evaluation

Performance of the AQ-TOP™ COVID-19 Rapid Detection Kit was evaluated using contrived clinical nasopharyngeal swab and sputum specimens. A total of 60 contrived positive specimens (30 contrived positive nasopharyngeal swab specimens and 30 contrived positive sputum specimens) and 60 negative specimens were tested (30 negative nasopharyngeal swab and 30 negative sputum specimens). Leftover individual unique clinical nasopharyngeal swab and sputum matrices were determined to be negative using the U-TOP™ COVID-19 Detection Kit (FDA EUA 27-Apr-2020) prior to spiking in the RNA.

SARS-CoV-2 viral genomic RNA (NCCP No. 43326. National Culture Collection for Pathogens) was spiked into the clinical matrices at various concentrations relative to the assay's LoD. Of the 30 contrived nasopharyngeal swab positive samples, 20 were spiked at concentrations equivalent to 2X LoD (14 copies/µL), and 10 were spiked with concentrations equivalent to 3X LoD (21 copies/µL). Of the 30 contrived sputum positive samples, 20 were spiked at 2X LoD (14 copies/µL) and 10 were spiked with concentrations of 3X LoD (21 copies/µL). The remaining 30 nasopharyngeal swabs and 30 sputums were tested as negative clinical samples.

Prepared samples were randomized and blinded, and RNA was extracted using the QIAamp DSP Virus Kit. Testing was performed in a total of two (2) RT-LAMP runs with one positive and one negative control included per run on the CFX 96 Real-Time PCR System. All negative samples were non-reactive and positive spiked samples at 2X and 3X LoD for both nasopharyngeal swabs and sputum showed 100% detection. Results of the study are summarized in Table 9.

Table 9. Clinical Evaluation with Contrived Nasopharyngeal Swab and Sputum Specimens

Specimen type	Concentration	Number of samples	Detection rate		Mean Ct	
			ORF1ab	IC	ORF1ab	IC
Nasopharyngeal swab	2X LoD	20	20/20	20/20	18.8	23.8
	3X LoD	10	10/10	10/10	17.4	24.9
	Negative	30	0/30	0/30	-	23.1
Sputum	2X LoD	20	20/20	20/20	19.1	23.2
	3X LoD	10	10/10	10/10	17.9	21.6
	Negative	30	0/30	30	-	23.1

An additional study was performed to evaluate the performance of the AQ-TOP™ COVID-19 Rapid Detection Kit testing individual, leftover, de-identified nasopharyngeal swab and sputum clinical specimens. A total of 35 positive specimens (20 nasopharyngeal swabs, 15 sputum samples) and 40 negative specimens (25 nasopharyngeal swabs, 15 sputum samples) were

analyzed on CFX 96 Real-time PCR system by AQ-TOP™ COVID-19 Rapid Detection Kit. Specimens were previously tested using the EUA authorized test, U-TOP™ COVID-19 Detection Kit, authorized on 27-Apr-2020.

Both positive percent agreement (PPA) and negative percent agreement (NPA) between the 2 assays for both specimen types were 100%. The results are summarized in Table 10 and Table 11.

Table 10. Performance of Nasopharyngeal Swabs When Compared to the U-TOP™ EUA Authorized Assay

Nasopharyngeal Swabs		Comparator Assay (U-TOP™ COVID-19 Detection Kit)		
		Positive	Negative	Total
AQ™-TOP COVID-19 Rapid Detection Kit Result	Positive	20	0	20
	Negative	0	25	25
	Total	20	25	45
Positive Agreement		100.0% (20/20); 83.89% - 100.00%*		
Negative Agreement		100.0% (25/25); 86.68% - 100.00%*		

Table 11. Performance of Sputum Specimens When Compared to the U-TOP™ EUA Authorized Assay

Sputum		Comparator Assay (U-TOP™ COVID-19 Detection Kit)		
		Positive	Negative	Total
AQ™-TOP COVID-19 Rapid Detection Kit Result	Positive	15	0	15
	Negative	0	15	15
	Total	15	15	30
Positive Agreement		100.0% (15/15); 79.62% - 100.00%*		
Negative Agreement		100.0% (15/15); 79.62% - 100.00%*		

Appendix A. FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method and instrument used were QIAamp DSP virus kit (Qiagen, Cat No.60704) and CFX 96 Real-Time PCR Detection System. The results are summarized in Table 12.

Table 12. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP Swab	$6x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

13. Trouble shooting

Problem	Cause	Solution
Fluorescence signal is not detected in all samples	Error of the PCR reaction	Review if any reagent was missed during the preparation process
	If the storage conditions of the kit are not appropriate, or the expiration date has expired	Repeat the test after checking the storage conditions and expiration date
Fluorescent signal is low in all samples	If the PCR reagents were not mixed correctly	Proceed with the test after review of PCR mix
	Long storage at room temperature or light exposure	Dispose the kit.
	If the expiration date has passed	Check the expiration date of the kit
Signal detection in Negative Control	If the PCR mixture or Negative control are contaminated	Discard and use new
	If the experiment place or the tool is contaminated	Check whether the test site or tool is contaminated. Repeat the experiment with new aliquots of all reagents
If there are different results in the same sample	Pipetting error	Check the pipette
	Cross contamination	Be careful with DNA splitting and repeat the test
	Contaminated 96-well plate	Test with a new 96-well plate
- SEASUN BIOMATERIALS Inc. guarantees all its products before the expiration date - Contact our A/S team if a problem not mentioned in this table has occurred		

14. Reference

1. Victor M Corman et al., Diagnostic detection of SARS-CoV-2 by real-time RT-PCR. Euro Surveill 2020. 25(3): 2000045
2. Leo Poon et al., Detection of 2019 novel coronavirus (SARS-CoV-2) in suspected human cases by RT-PCR. www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf.
3. Mary Johnson. Wuhan 2019 Novel Coronavirus – 2019–nCoV. MATER METHODS. 2020. 10:2867
4. Zheng-Li Shi et al., Discovery of a novel coronavirus associated with the recent pneumonia outbreak in 2 humans and its potential bat origin. Nature. 2020 579(7798): 270-273
5. Naganori Nao et alk., Detection of second case of SARS-CoV-2 infection in Japan. NIID. 2020.
6. 2019-Novel coronavirus (SARS-CoV-2) real-time rRT-PCR panel primers and probes. 2020. US Centers for Disease Control and Prevention.
7. SARS-CoV-2 detection real-time RT-PCR protocol. 2020. KCDC v1.5

15. Symbols

	Catalogue Number		Expiration Date
	Temperature limitation (Storage temperature)		Manufacturer
	In vitro Diagnostic Medical Device		Lot number
	Do Not Reuse (For single use only)		

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