AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2

Instructions for Use

Intended Use:

The AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2 is an RT-qPCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in nasopharyngeal (NP) and oropharyngeal (OP) swabs from individuals with signs and symptoms of infection who are suspected of COVID-19. 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, or by similarly qualified non-U.S. laboratories.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in nasopharyngeal and oropharyngeal swabs during the acute phase of infection. Positive results are indicative of active infection. 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 2019-nCoV 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 AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2 is intended for use by qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Test Principle:

Nucleic acids are isolated and purified from nasopharyngeal and oropharyngeal swabs using a previously FDA cleared nucleic acid extraction system. Sample input and elution volumes are system dependent. The purified nucleic acid is reverse transcribed into cDNA by combining the nucleic acid with the AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2 master mix. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle.

Materials Provide and Storage:

Concentration: 2X
Storage and Handling: Store at -20˚C upon arrival
Ordering Information:

Item Number	Total Volume Received	Quantity Received	Total number of reactions which can be obtained when using the following reaction sizes	
			20µL Reactions	10µL Reactions
AzureSeq-200	SB-ITMP-MM-100-PC SB-RT-200-PC SB-CPPM-100uL-PC SB-NFW-350uL-PC SB-CVNC-150-PC SB-CVPC-150-PC	SB-ITMP-MM-100-PC: 2 x 1mL SB-RT-200-PC: 1 x 100µL SB-CPPM-100uL-PC: 2 x 100µL SB-NFW-350uL-PC: 2 x 350µL SB-CVNC-150-PC: 1 x 150µL SB-CVPC-150-PC: 1 x 150µL	200	400

Workflow:

Protocol for One-Step SARS-nCoV-2 Detection: The following reaction set up cycling conditions are recommended but can vary depending on the template and primers being used. Reaction volumes are to be determined by the end user. Recommended protocols for 20µL, 10µL, or 5µL reactions are listed on the following pages.

Instructions for setting up 20µL reactions:

1. Completely thaw the CoVi Primer/Probe mix 3 (brown tube/cap) by setting on ice for ~30 minutes. Once thawed, briefly centrifuge to collect at the bottom of the tube, **then add 384µL of nuclease free water to the tube**. Mark the tube with water added.
2. Vortex the tube at max speed for 10 seconds to mix, then spin down briefly to collect at bottom of tube.
3. Proceed to master mix setup as shown below in a clean room or designated setup area:

Reaction set-up for 20µL reaction volume Table 1:

Component	Volume 1 reaction	Volume 100 reactions (96-well plate)	Final Concentration
2X InhibiTaq Multiplex qPCR MasterMix	10 µl	1000 µl	1X
RTScript™, 200U/µL	0.5 µl	50 µl	5 units/µl
Diluted Primer/Probe Mix	4.5 µl	450 µl	1X

4. Mix the master mix by pipetting up and down repeatedly with pipette set to volume of 2X master mix added, or by capping the tube, vortexing briefly, and spinning down briefly to collect mix.
5. Distribute **15 µl** of the master mix using an appropriate pipette to all wells of a plate that will be used.
6. Add **5 µl** of sample, positive control, or negative control to appropriate wells.
7. Seal the plate, vortex briefly or flick to mix; spin down in a centrifuge to collect the mixed samples.
8. Place the plate into the designated real-time machine and run the following program:

Thermal cycling conditions:

Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
RT Incubation	1	1	50°C	15 minutes
Enzyme Activation	2	1	95°C	2 minutes
Amplification**	3	45	95°C	3 seconds
			60°C**	30 seconds

**Collect fluorescence during annealing/extension phase (55°C) step on FAM, HEX, and ROX channels (or equivalent channels).

Instructions for setting up 10µL reactions:

1. Completely thaw the CoVi Primer/Probe mix 3 (brown tube/cap) by setting on ice for ~30 minutes. Once thawed, briefly centrifuge to collect at the bottom of the tube, **then add 384µL of nuclease free water to the tube**. Mark the tube with water added.
2. Vortex the tube at max speed for 10 seconds to mix, then spin down briefly to collect at bottom of tube.
3. Proceed to master mix setup as shown below in a clean room or designated setup area:

Reaction set-up for 10µL reaction volume Table 2:

Component	Volume 1 reaction	Volume 100 reactions (96-well plate)	Final Concentration
2X InhibiTaq Multiplex qPCR MasterMix	5 µl	500 µl	1X
RTScript™, 200U/µL	0.25 µl	25 µl	5 units/µl
Diluted Primer/Probe Mix	2.25 µl	225 µl	1X

4. Mix the master mix by pipetting up and down repeatedly with pipette set to volume of 2X master mix added, or by capping the tube, vortexing briefly, and spinning down briefly to collect mix.
5. Distribute **7.5 µl** of the master mix using an appropriate pipette to all wells of a plate that will be used.
6. Add **2.5 µl** of sample, positive control, or negative control to appropriate wells.
7. Seal the plate, vortex briefly or flick to mix; spin down in a centrifuge to collect the mixed samples.
8. Place the plate into the designated real-time machine and run the following program:

Thermal cycling conditions:

Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
RT Incubation	1	1	50°C	15 minutes
Enzyme Activation	2	1	95°C	2 minutes
Amplification**	3	45	95°C	3 seconds
			60°C**	30 seconds

**Collect fluorescence during annealing/extension phase (55°C) step on FAM, HEX, and ROX channels (or equivalent channels).

Instructions for setting up 5µL reactions:

1. Completely thaw the CoVi Primer/Probe mix 3 (brown tube/cap) by setting on ice for ~30 minutes. Once thawed, briefly centrifuge to collect at the bottom of the tube, **then add 55µL of nuclease free water to the tube**. Mark the tube with water added.
2. Vortex the tube at max speed for 10 seconds to mix, then spin down briefly to collect at bottom of tube.
3. Proceed to master mix setup as shown below in a clean room or designated setup area:

Reaction set-up for 5µL reaction volume Table 3:

Component	Volume 1 reaction	Volume 100 reactions (96-well plate)	Final Concentration
2X InhibiTaq Multiplex qPCR MasterMix	2.5 µl	250 µl	1X
RTScript™, 200U/µL	0.125 µl	12.5 µl	5 units/µl
Diluted Primer/Probe Mix	0.375 µl	37.5 µl	1X

4. Mix the master mix by pipetting up and down repeatedly with pipette set to volume of 2X master mix added, or by capping the tube, vortexing briefly, and spinning down briefly to collect mix.
5. Distribute **3 µl** of the master mix using an appropriate pipette to all wells of a plate that will be used.
6. Add **2 µl** of sample, positive control, or negative control to appropriate wells.
7. Seal the plate, vortex briefly or flick to mix; spin down in a centrifuge to collect the mixed samples.
8. Place the plate into the designated real-time machine and run the following program:

Thermal cycling conditions:

Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
RT Incubation	1	1	50°C	15 minutes
Enzyme Activation	2	1	95°C	2 minutes
Amplification**	3	45	95°C	3 seconds
			60°C**	30 seconds

**Collect fluorescence during annealing/extension phase (55°C) step on FAM, HEX, and ROX channels (or equivalent channels).

Risk and Safety Information:

Safety Data Sheets are available online at www.seqonce.com

The user should carefully read all warnings, instructions or Safety Data Sheets provided by the supplier for any additional materials or chemicals required for the use of the AzureSeq One-Step Universal RT-qPCR Kit SARS-CoV-2. The user should also follow general safety precautions when handling biohazards, chemicals and other materials.

General Precautions:

1. Treat all samples, materials and instrumentation as potentially infectious.
2. Avoid potential contamination by employing good laboratory practices, wearing proper personal protective equipment, and decontaminating workspaces before and after use.
3. All instruments must be maintained and operated according to manufacturer's instructions.
4. Dispose of waste according to state and local regulations.
5. Only use DNase and RNase free consumables.
6. Do not use reagents beyond their expiration.

Manufacturing Information:

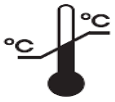
Empirical Bioscience
2007 East Castle Drive, SE
Grand Rapids, MI 49508

Support:

Phone: +1-626-460-3050
Email: info@seqonce.com

Explanation of Symbols:

Symbol	Definition
IVD	***In vitro*** **diagnostic medical device**

	**Lot Number**
	**Manufacturer**
	**Date of Manufacture**
	**Storage & Transport Conditions**
	**Consult instructions for use**
	**Use by Date**