

## AzureSeq Plus One-Step Universal RT-qPCR Kit, SARS-CoV-2, Influenza A, Influenza B Kit

### Instructions for Use – Extracted RNA Method

#### Intended Use:

The AzureSeq Plus One-Step Universal RT-qPCR Kit SARS-CoV-2, Influenza A, Influenza B is an RT-qPCR test intended for the qualitative detection of nucleic acid from 2019-nCoV, Influenza A, and Influenza B, in nasopharyngeal (NP) and oropharyngeal (OP) swabs from individuals with signs and symptoms of infection who are suspected of COVID-19 or influenza. 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, Influenza A RNA, and Influenza B RNA. RNA from 2019-nCoV-2, Influenza A, and Influenza B 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 or Influenza 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 Plus One-Step Universal RT-qPCR SARS-CoV-2, Influenza A/B kit 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 Plus One-Step Universal RT-qPCR SARS-CoV-2, Influenza A, Influenza B kit 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. Extracted viral RNA is reverse transcribed into cDNA with the AzureSeq Plus One-Step Universal RT-qPCR Kit SARS-CoV-2, Influenza A/B master mix. Sample input and elution volumes are system dependent. Probes present in the master mix anneal 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 specific PCR cycles.



**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
ASD4-200 Plus	SB-ITMP-MM-100-PC SB-DRT-200-PC SB-CPPM4-100uL-PC SB-NFW-350uL-PC SB-CVNC-150-PC SB-CVPC4-150-PC	SB-ITMP-MM-100-PC: 2 x 1mL SB-DRT-200-PC: 1 x 275µL SB-CPPM4-100uL-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

**Workflow:**

**Protocol for One-Step SARS-nCoV-2, Influenza A, Influenza B Detection:** The following reaction set up cycling conditions are recommended but can vary depending on the template and primers being used. Reaction volumes are optimized at 20ul.

**Detection:**

Target	Dye
SARS-CoV-2 N1	FAM
Influenza A M1	Quasar 670
Influenza B NS2	HEX
RnaseP	ROX

- HEX can be replaced by VIC or JOE if not present on the program.
- Texas Red can be replaced by ROX if not present on the program.
- Cy5 can be replaced by Quasar 670 if not present on the program.
- Select all four dyes for each labeled well

**Instructions for setting up 20µL reactions:**

**Use With Extracted RNA**

**RT-qPCR Master Mix and Reagent Setup**

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.
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:

### Master Mix 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
Direct RTScript Mix	1.25 µl	125 µl	1X
20X Primer/Probe Mix	1.0 µl	100 µl	1X
Nuclease Free Water	2.75 µl	275 µl	n/a

\*vortex to mix prior to use

- Mix the master mix created in step 3 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.
- Distribute **15 µl** of the master mix using an appropriate pipette to all wells of a plate that will be used.
- Add **5 µl** of purified RNA sample, 5 µl positive control, or 5 µl negative control to appropriate wells.
- Seal the plate, vortex briefly or flick to mix; spin down in a centrifuge to collect the mixed samples.
- Place the plate into the designated real-time machine and run the following conditions:

#### 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	40 (Minimum)	95°C	3 seconds
			60°C**	30 seconds

\*\*Collect fluorescence during annealing/extension phase (60°C) step on FAM, HEX, Quasar 670 (Cy5) and ROX channels (or equivalent channels).

##### Rapid Cycling Conditions- Under Review

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
RT Incubation	1	1	50°C	5 minutes
Enzyme Activation	2	1	95°C	1 minute
Amplification**	3	40 (Minimum)	95°C	1 second
			60°C**	1 second

\*\*Collect fluorescence during annealing/extension phase (60°C) step on FAM, HEX, Quasar 670 (Cy5) and ROX channels (or equivalent channels).

#### Interpretation of Results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Ct values <40 are interpreted as positive by the user for the BioRad CFX96 and QuantStudio 3, 5, and 7 Pro.

SARS-CoV-2 (FAM)	Influenza A (Qua670)	Influenza B (HEX)	RNaseP (ROX)	Result Interpretation	Action
+	-	-	+/-	Positive SARS-CoV-2	Report results to the healthcare provider and appropriate public health agencies
-	-	-	-	Invalid	Repeat test. If still invalid collect another specimen. If another specimen is not available, report to the healthcare provider
-	-	-	+	SARS-CoV-2, Influenza A, Influenza B not detected	Report results to healthcare provider
-	+	-	+/-	Positive Influenza A	Report results to the healthcare provider and appropriate public health agencies
-	-	+	+/-	Positive Influenza B	Report results to the healthcare provider and appropriate public health agencies
+	+	-	+/-	Positive SARS-CoV-2 Positive Influenza A	Report results to the healthcare provider and appropriate public health agencies
-	+	+	+/-	Positive Influenza A Positive Influenza B	Report results to the healthcare provider and appropriate public health agencies
+	-	+	+/-	Positive SARS-CoV-2 Positive Influenza B	Report results to the healthcare provider and appropriate public health agencies
+	+	+	+/-	Positive SARS-CoV-2 Positive Influenza A Positive Influenza B	Report results to the healthcare provider and appropriate public health agencies

**Warning: This assay is designed and warranted (only) for a 20 µl reaction volume.**

Reducing reaction volume may decrease sensitivity, reduce specificity, and/or impact detection.

### Risk and Safety Information:

Safety Data Sheets are available online at [www.seqonce.com](http://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:**





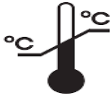




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**Explanation of Symbols:**

Symbol	Definition
	<i>In vitro</i> diagnostic medical device
	Lot Number
	Manufacturer
	Date of Manufacture
	Storage & Transport Conditions

	<b>Consult instructions for use</b>
	<b>Use by Date</b>