

AzureSeq Direct One-Step Universal RT-qPCR Kit

SARS-CoV-2 N501Y Variant Assay

Instructions for Use

Intended Use:

The AzureSeq Direct One-Step Universal RT-qPCR Kit SARS-CoV-2 N501Y Variant Assay 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 Direct One-Step Universal RT-qPCR Kit SARS-CoV-2 N501Y Variant Assay 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 Direct One-Step Universal RT-qPCR Kit SARS-CoV-2 N501Y Variant Assay is for Research Use Only and requires an LDT for use by CLIA laboratories, or similar for qualified non-U.S. laboratories.

Test Principle:

Viral transport media containing sample is heat-inactivated at 95C. Viral RNA is reverse transcribed into cDNA by combining the nucleic acid with the AzureSeq Direct One-Step Universal RT-qPCR Kit SARS-CoV-2 501Y Variant Assay 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. This test assesses polymorphisms for SARS-CoV-2 variant N501Y (A23063T), differentiating between A (wild type) and T (variant), and presence of nucleocapsid N gene (N1). RNaseP is used as a positive control for sample integrity.



Materials Provide and Storage:

Concentration: 2X

Storage and Handling: Store at -20°C upon arrival

Ordering Information:

Item Number	Total Volume Received	Quantity Received
AS-501-200	SB-ITMP-MM-100-PC SB-DRT-200-PC SB-CPPM-501-4-100-PC SB-NFW-350uL-PC SB-CVNC-150-PC SB-CVPC-150-PC	SB-ITMP-MM-100-PC: 2 x 1mL SB-DRT-200-PC: 1 x 250µL SB-CPPM-501-4-100-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

Workflow:

Protocol for Direct One-Step SARS-nCoV-2 N501Y Variant 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. The recommended protocol for 20µL reaction is listed on the following pages.

Detection:

- Dyes in the Multiplex Primers/Probe: N1 (FAM), N501Y Wildtype (SUN), N501Y variant (Cy5), RnaseP (Texas Red)
 - SUN can be replaced by HEX or VIC if not present on the program.
 - Texas Red can be replaced by ROX if not present on the program.
- Select all four dyes for each labeled well

Instructions for setting up 20µL reactions:

Direct RNA Extraction

(Recommended for use only with OP or NP swabs in Viral Transport Media (VTM) from the following Manufacturers: Copan, Puritan, CDC, Saline, PBS, Mawi EL)

1. Obtain swabbed OP/NP material in VTM.
2. Transfer **100-200µL** of swabbed OP/NP VTM material into compatible DNase/RNase free tube.
3. Heat sample for **5 minutes at 95°C**.
4. Following incubation, briefly spin heated sample to collect material at the bottom of the tube. Store on ice until needed. The sample is now ready to be added to the RT-qPCR reaction (see following steps).

Using Previously Isolated RNA: Skip the Direct RNA Extraction step (above) and use 5ul of extracted RNA (sample) in Tris or H2O, in Step 6 (below).

RT-qPCR Master Mix and Reagent Setup

1. Completely thaw the 2x InhibiTaq MM and CoVi N501Y Primer/Probe mix 4 (brown tube/cap) by setting on ice for ~30 minutes. Reagents can be thawed at room temperature for ~5 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 combine in a 1.5mL Eppendorf tube, in the order shown below, in a clean room or designated setup area:

Component	Volume 1 reaction (µl)	Volume 100 reactions (96-well plate (µl))
Sterile Water	2.75	275
CoVi N501Y Primer/Probe Mix 4	1	100
2X InhibiTaq Multiplex qPCR	10	1000
Direct RTScript™, 200U/µL	1.25	125

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	40-45	95°C	3 seconds
			60°C**	30 seconds

**Collect fluorescence during annealing/extension phase (60°C) step on FAM, SUN, CY5, and ROX 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, SUN, CY5, and ROX channels.

Interpretation of Results:

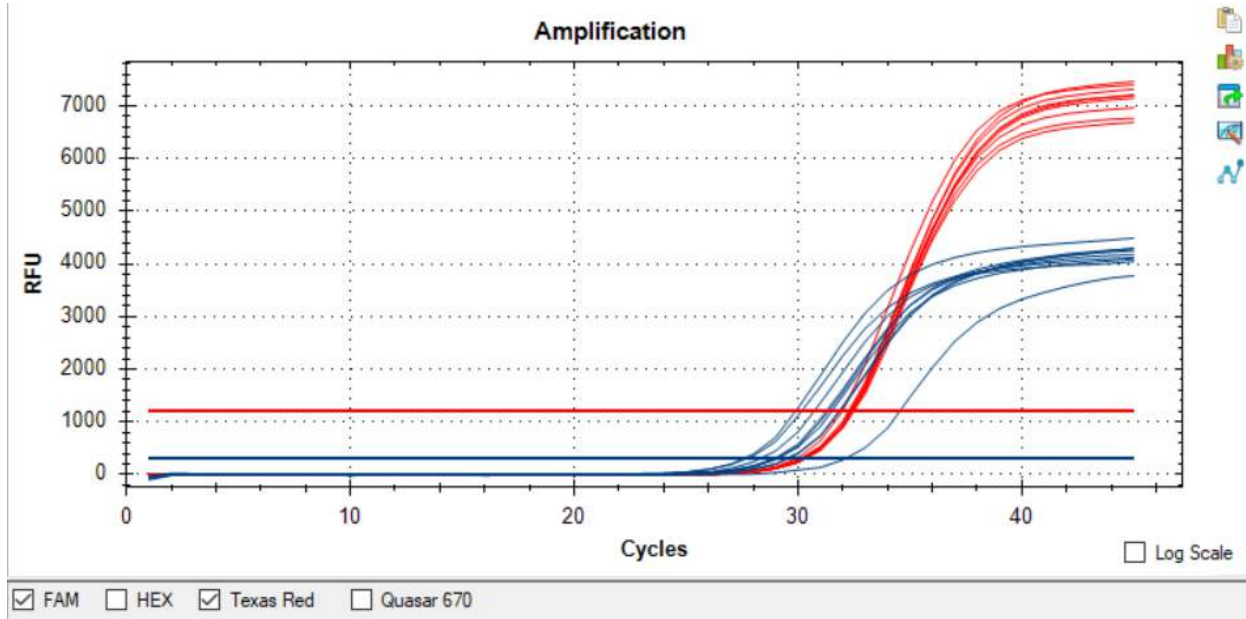
All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Ct values <40 are interpreted as positive by the user for the BioRad CFX96 and QuantStudio 3, 5, and 7 Pro.

SARS-CoV-2 N1 (FAM)	SARS-CoV-2 N501 Wildtype (SUN)	SARS-CoV-2 N501Y Variant (Cy5)	RNaseP (Texas Red)	Result Interpretation	Report	Actions
Any Positive			+/-	2019-nCov detected	Positive 2019-nCov	Report results to the healthcare provider and appropriate public health agencies
+	+	-	+/-	2019-nCov detected Presumed N501 Wildtype	Positive 2019-nCov	Report results to the healthcare provider and appropriate public health agencies
+	-	+	+/-	2019-nCov detected Presumed N501Y Variant	Positive 2019-nCov	Report results to the healthcare provider and appropriate public health agencies
-	+	+	+/-	2019-nCov detected Inconclusive Variant ID	Positive 2019-nCov	Report results to the healthcare provider and appropriate public health agencies
-	-	-	-	Invalid Result	Invalid	Report results to healthcare provider
-	-	-	+	2019-nCov not detected	Negative	Report results to healthcare provider

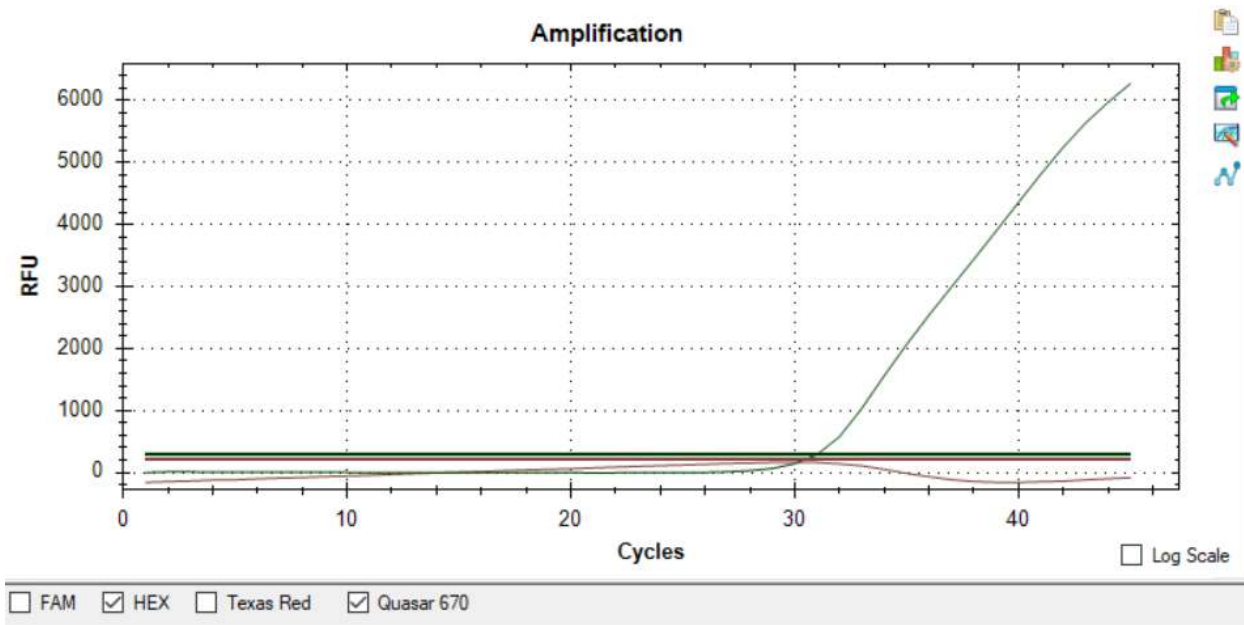
Warning: This assay is designed and warranted (only) for a **20 µl** reaction volume.

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

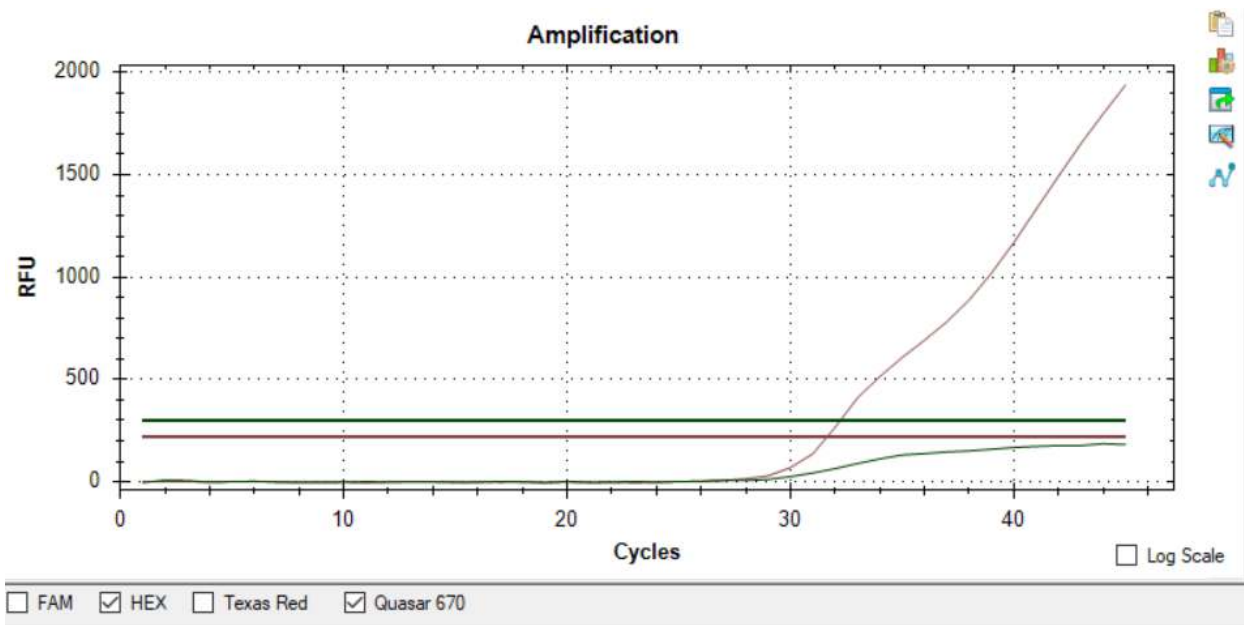
Analysis Using BioRad Maestro Software:



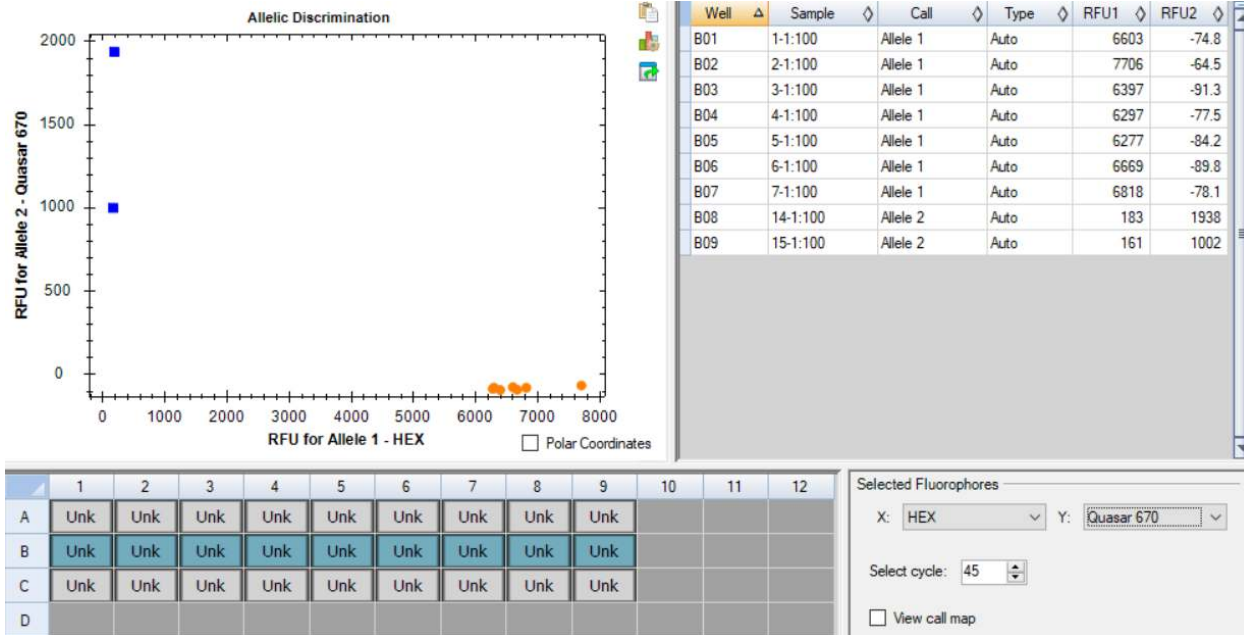
Review the FAM (N1) and Texas Red (RNaseP) fluorophores to confirm sample validity with the RNaseP IPC (internal PCR control) result. Review N1 result to identify positive samples (note if N1 amplification is present but RNaseP is absent, result is still valid). For all samples that are identified as 2019-nCoV not detected, no further review of the genotyping assay is necessary.



N501Y Wildtype result. HEX (wild type) shows amplification while Quasar670 (N501Y variant) shows no amplification.



N501Y variant result. HEX (wild type) shows no amplification while Quasar670 (N501Y variant) shows amplification.



Allelic Discrimination for genotyping on the CFX Maestro software. Selected fluorophores has been configured for HEX (N501Y wildtype) and Quasar670 (N501Y variant). This is displayed on the allelic discrimination plot for X axis as the wildtype while Y axis as the variant. The table in the top right also displays the allele call for the genotyping assay.

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 Direct 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

	Consult instructions for use
	Use by Date