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: 4X
Storage and Handling: Store at -20^{0}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
QSP-KIT-200	ITP-MM-200-PC: 1mL, RT-200-PC: 100uL, DTT-100-200uL-PC: 200uL	ITP-MM-200-PC: 1x1mL, RT-200-PC: 1x100uL, DTT-100-200uL-PC: 1x200uL	200	400
QSP-KIT-1000	ITP-MM-200-PC: 5mL, RT-200-PC: 500uL, DTT-100-200uL-PC: 1000uL	ITP-MM-200-PC: 5x1mL, RT-200-PC: 5x100uL, DTT-100-200uL-PC: 5x200uL	1000	2000
QSP-KIT-2000	ITP-MM-200-PC: 10mL, RT-200-PC: 1000uL, DTT-100-200uL-PC: 2000uL	ITP-MM-200-PC: 10x1mL, RT-200-PC: 10x100uL, DTT-100-200uL-PC: 10x200uL	2000	4000
QS-SR-100***	5X RTScript Buffer-1x0.4mL, 10mM dNTP Mix-1x0.1mL, 100mM DTT Solution-1x0.1mL, 100µM Oligo-(dT)20 primer-1x0.05mL, 100µM Random Hexamers-1x0.05mL, 40 Units/uL Rnase Inhibitor-1x0.01mL, 10ng/uL Positive Control RNA-1x0.01mL, Rnase-free Water-1x1.2mL		100	200
EVA-300uL****	EvaGreen Dye, 20X in Water; EVA: 2x150uL		200	400
ROX-300uL****	ROX Reference Dye, 20X in Water; ROX: 2x150uL		Determined my volume required in assay by machine	Determined my volume required in assay by machine

***This item can be purchased separately to perform a Two-Step Protocol: First Strand cDNA Synthesis followed by Amplification.
****This item can be purchased separately if dyes are required for qPCR assays.

Workflow:

Protocol for One-Step: The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

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

Component	Volume	Final Concentration
4X InhibiTaq Plus HotStart qPCR MasterMix	5 µl	1X
RTScript™, 200U/µL#	0.5 µl	5 units/µl
Target Specific Primers and Probe	1µl	1X
100mM DTT#	1µl	5mM
Sample	X µl	100 ng to 1 pg
EvaGreen Dye, 20X in Water (if required)	1 µl	1X
ROX reference dye, 25µM (See Table 2)	See Table 2	See Table 2

Nuclease Free Water to volume	X µl	N.A.
Total	20 µl	

#Reactions need to be placed in cycler immediately following completion to maintain the integrity of the DTT and the RTScript™.

Table 2: ROX concentration recommendation for different instrument

Type	Instrument		ROX final concentration
	Company	Instrument Name	
No ROX	Roche	LightCycler 480, LightCycler 2.0	None
	BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, Chromo4, MJ Opticon, Option2, MiniOpticon	
	Qiagen	Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000	
	Illumina	Eco RealTime PCR System	
	Eppendorf	Mastercycler realplex	
	Cepheid	SmartCyler	
Low ROX	ABI	7500, 7500 Fast	30nM
	Stratagene	MX4000P, MX3000P, MX3005P	
High ROX	ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus, Viia7	300nM

Thermal cycling conditions:

Table 3: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
UNG Incubation	1	1	25°C	2 minutes
RT Incubation	2	1	55°C	15 minutes
Enzyme Activation	3	1	95°C	2 minutes
Amplification**	4	40	95°C	10 seconds
			60°C	60 seconds

**Temperature and Holding Time will be based off primer set used.

Protocol for Two-Step: The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

Step 1A: cDNA synthesis without denaturation. General guidelines per 20µl reaction for setup without sample denaturation include, See Table 4

Table 4: cDNA synthesis without denaturation.

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 20µL
RNA template	-	Total RNA: 10 pg-5 µg or mRNA: 10pg-500ng	X µL
Primer	100µM	Gene specific primer: 10-20pg (50-100ng) Oligo-dT$_{20}$ primer or random: 50pmol	0.1-0.2 µL 0.5µL

5X RTScript Buffer	5X	1X	4µL
dNTP Mix	10mM	500µM each	1 µL
DTT stock solution	100mM	5mM	1 µL
RNase Inhibitor	40 units / µL	20 units	0.5 µL
RTScript™ Reverse Transcriptase	200 units / µL	100 units	0.5 µL

Use reaction mix immediately to preserve the integrity of the enzyme.

Step 1B (optional): cDNA synthesis with denaturation.

- First, prepare the Template/Primer mix using the Table 5. Incubate the mix for 5 min at 65-70°C.

Table 5: Template/Primer Mix

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 10µL
RNA template	-	Total RNA: 10 pg-5 µg or mRNA: 10pg-500ng	X µL
Primer	100µM	Gene specific primer: 10-20pg (50-100ng) Oligo-dt_{15-25} primer or random: 50pmol	0.1-0.2 µL 0.5µL

- Second, prepare a Reaction mix using Table 6.

Table 6:

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 10µL
5X RTScript Buffer	5X	1X	4µL
dNTP Mix	10mM	500µM each	1 µL
@DTT stock solution	100mM	5mM	1 µL
%RNase Inhibitor	40 units / µL	20 units	0.5 µL
RTScript™ Reverse Transcriptase	200 units / µL	100 units	0.5 µL

@Adding up to 5mM DTT may increase the yield and is recommended for individual optimization %Addition of 20-40 Units of RNase inhibiter per assay is recommended when using low amounts of starting RNA. #100 Units of enzyme is recommended for standard assays, but increased transcription levels may be achieved with increasing the amount of enzyme up to 200 units. ***Use reaction mix immediately to preserve the integrity of the enzyme.***

- Third, Add 10µl of Reaction Mix to 10uL of Template/primer mix and pipette gently up and down on ice.

Step 2: Incubation
Gene Specific primers: Incubate Reaction Mix for 30-60min at 50°C.
Oligo-dT or Random primers: Incubate Reaction Mix for 10min at 42°C followed by 30-60min at 50°C.

Step 3 (optional): Heat inactivation
Heat the mixture for 70°C for 10 min to inactivate Reverse Transcriptase.

Step 4(optional): RNA removal
Add 2 Units DNase-free RNase and incubate at 37°C for 20min.

The cDNA can now be used as a template in qPCR.

Reaction set-up for 20uL reaction volume

Table 7: Reaction Set Up

Component	Volume	Final Concentration
4X InhibiTaq Plus HotStart qPCR	5 µl	1X
Target Specific Primers and Probe	1µl	1X
100mM DTT#	1µl	5mM
cDNA Template from Two-Step Protocol	X µl	100 ng to 1 pg
EvaGreen Dye, 20X in Water (if required)	1 µl	1X
ROX reference dye, 25µM (See Table 2)	See Table 2	See Table 2
Nuclease Free Water to volume	X µl	N.A.
Total	20 µl	

#Reactions need to be placed in cycler immediately following completion to maintain the integrity of the DTT.

Thermal cycling conditions:

Table 8: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
UNG Incubation	1	1	25°C	2 minutes
RT Incubation	2	1	55°C	15 minutes
Enzyme Activation	3	1	95°C	2 minutes
Amplification**	4	40	95°C	10 seconds
			60°C	60 seconds

**Temperature and Holding Time will be based off primer set used.

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:

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Support:

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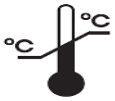
Explanation of Symbols:

Symbol	Definition
	***In vitro*** **diagnostic medical device**
	**Lot Number**
	**Manufacturer**
	**Date of Manufacture**
	**Storage & Transport Conditions**

	**Consult instructions for use**
	**Use by Date**