AstraArt COVID 19 qPCR test

PRINCIPLE

AstraArt COVID 19 qPCR test is a real-time reverse transcription polymerase chain assay for the qualitative detection of nucleic acid from the SARS-CoV2 in human nasopharyngeal swab extracts from individuals who are suspected of COVID-19. The SARS-CoV-2 primer and probe set(s) are designed to detect specific sequences of **N gene and ORF1ab Gene** of the **SARS-CoV-2** genome and Internal Control primer and probe is designed to detect **β2M gene.**

INSTRUCTIONS FOR USE

Avoid repeated freeze-thaw of reagents.

PACKAGE CONTENTS

Description	Specification	Quantity for 100 tests
Solution A	PCR Amplification Mix	500 µL x 1 tube
Solution B	Target Specific Primer Probe mix	1000µL x 1 tube
Negative control	Purified water	500 µL x 1 tube
Positive control	DNA positive control	200 µL x 1 tube

Catalogue Number	Description
AG/COVID-19/21/03	Applicable for COVID-19 testing

STORAGE & STABILITY

- All the reagents should be stored at - 20°C (±5). Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. Avoid repeated freeze/thaw cycles for reagents not more than 10 times
- Transportation under ambient temperature, but not higher than 25° C (up to 10 days); under + 2 – +8°C temperature up to 30 days.

SAMPLE REQUIREMENTS

- It is ideal to carry out extraction protocol with fresh samples.
- Extraction can also be performed with samples stored at 2-8°C for short period of time.
- For long-term storage, freezing at −20 to −80°C is recommended.
- Repeated freezing and thawing should be avoided.
- Transport the specimens in ice/ sealed with ice / sealed foam box with ice.

SAMPLE COLLECTION

Specimen Collection: Swab from posterior nasopharyngeal wall should be collected. Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing viral transport media.
Storage: If specimens are not shipped or processed immediately, it is acceptable to store specimens at 2-8°C for up to 24 hours after collection. If a delay in testing or shipping is expected to exceed 24 hours, specimens can be stored at -70°C or below until used.

ASSAY PROCEDURE

Nucleic acids are isolated from the collected swabs using Nucleic acid purification system. The purified nucleic acid is directly amplified using the AstraArt COVID 19 qPCR test on the Real-time PCR Instrument system. 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 by the Real-time PCR Instrument system.

a. Preparation of Real-time PCR reagents: Briefly Centrifuge all the reagents. Prepare the reagents according to the table below. The final volume is calculated by multiplying the number of samples by the volume of each component in Table 1.

Table 1: Components of reaction mix:

Component	Volume (µL) per reaction
PCR Mix	5
Primer Mix	10
Template RNA/Positive control/ Negative control	10
Total volume	**25**

b. Mix the reaction master mix except the template, and spin-down briefly. Aliquot 15 µL of the master mix into each well of 96-well plate and add 10 µL of the template RNA/Positive control/Negative control accordingly. Seal the plate and spin-down briefly. Run the Protocol immediately on the Real-time PCR instrument with following cycling conditions in Table 2.

Table 2: Cycling Conditions:

Steps	Temperature° C	Time	Fluorescence data collection	Cycle
1 (cDNA Synthesis)	55	10 minutes		1
2 (Initial Denaturation)	95	15 minutes		1
3 (PCR Cycling) Stage 2	95	05 seconds	Fluorescence data collected	40
	60*	15 seconds		
	67	15 seconds		

ORF1ab Gene uses FAM Channel, N gene uses VIC channel, and endogenous β 2M gene uses Texas red channel.
***Fluorescence is measured at 60°C in PCR Cycling Stage 2.**
Note: Please select "None" in both Passive reference and Quencher. Depending on the Ramp rate of the PCR machine used the run times may vary.

c. Interpretation of Results:

- Total number of cycles for annealing and extension is 40 and florescent data is collected for 40 cycles.
- Interpret the values for unknown samples based on the observations as described in the following table. There should be no amplification signal in negative control. Endogenous IC should show signal in all the extracted samples to identify possible PCR Inhibitions. ≤38 Ct of unknown samples should be considered for result interpretation.
- Ct Values between 35-38 cycles are presumptive positive (PP).
- Ct value above 38 cycles is considered negative.

Table 3: Conclusion:

COVID-19 Result	N gene (VIC)	ORF1ab gene (FAM)	B2M gene (Texas Red)	Conclusion
***Positive Control**	+	+	+	Valid
***Negative Control**	-	-	-	Valid
Sample	+	+	+	COVID-19 Positive
Sample	-	-	+	COVID- 19 Negative
Sample	-	PP	+	Presumptive positive, Repeat the test with another kit.
Sample	PP	-	+	
Sample	PP	PP	+	
Sample	-	-	-	Possible inhibition of PCR/No sample collected

Analytical Sensitivity of the assay: The Lower Detection Limit (LOD) of the AstraArt COVID 19 qPCR test is defined as 10 copies/reactions.

LIMITATION OF THE PROCEDURE

- The use of this assay as an in vitro diagnostic and is limited to laboratories that are certified to perform high complexity tests.
- This kit is used for qualitative detection of SARS-CoV-2 RNA from specimens. The results do not reflect the viral load in the original specimen.
- The specimen 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 and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure.
- Amplification and detection of SARS-CoV-2 with AstraArt COVID 19 qPCR test has only been validated with Real-Time PCR instruments

WARNING & PRECAUTIONS

- Specimen collection should be done in the acute phase of illness
- Do not use the product if there is evidence of leakage.
- Adhere to standard procedures and published protocols for sample collection, processing, and disposal.

SYMBOLS:

Do not use if package is damaged

CE mark of Conformity

Refer to the instructions

ISO

AstraGene FZ LLC, Office No. 208 – 209, Dubai Science Park Building, Dubai, United Arab Emirates, +971-4-8781222, contact@astragene.com