AstraArt FLU/RSV/COVID 19 qPCR Kit

PRINCIPLE

AstraArt FLU/RSV/COVID-19 qPCR Kit is a is an inhibitor tolerant real-time polymerase chain reaction assay for the qualitative detection of RNA from Influenza, Human Respiratory Syncytial Virus (RSV) and SARS-CoV2 in respiratory specimens from individuals suspected of respiratory infections. The assay is a combination of the latest advanced buffer chemistry, PCR enhancers and stabilizers along with antibody-mediated hot-start polymerase, dNTPs and MgCl2. This assay has been designed for highly reproducible, accurate results in the presence of inhibitors, making it ideal for detection of Influenza (Flu A or B), Human Respiratory Syncytial Virus (RSV A or B), and SARS CoV2 (COVID-19).
The primer and probe set(s) are designed to detect specific sequences of **M/NS gene for Influenza A/B, NS/G gene for RSV A/B, N gene and E gene** for **SARS-CoV2** genome and Internal Control (RNaseP) primer and probe is designed to detect **Human housekeeping gene.**

INSTRUCTIONS FOR USE

Avoid repeated freeze-thaw of reagents.

PACKAGE CONTENTS

Description	Specification	Quantity for 100 tests
qPCR Master Mix	qPCR amplification Mix	1000ul x 1 tube
Primer mix	Target Specific Primer Probes	500ul x 1 tube
Negative control	Purified water	100ul x 1 tube
Positive control	DNA positive control	100ul x 1 tube

Catalogue Number	Description
AG/FRC /22/01	Applicable for testing Respiratory infection

STORAGE &STABILITY

- All the reagents should be stored at -20 ℃. Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. Avoid repeated freeze/thaw cycles for reagents.

SAMPLE REQUIREMENTS

- It is ideal to carry out protocol with fresh samples or extracted nucleic acid from stored samples.
- Extraction can also be performed with samples stored at 2-8℃ 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 isolated from the collected swab is directly amplified using the AstraArt FLU/RSV/COVID-19 qPCR Kit 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. Prepare the reagents according to the table below.

Table 1: Components of reaction mix:

Component	Volume (µℓ) per reaction
qPCR Master Mix	10
Primer Mix	5
Sample/RNA/ Positive control/ Negative control	10
Total volume	**25**

b. Seal the tubes, gentle mix 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	Cycle
1	50	3 minutes	1
2	95	2 minutes	1
3	95	5 seconds	35
4	60*	25 seconds	
FLU -TexasRed (TxRd), RSV- Cy5, COVID-19 N gene -FAM, COVID-19 E gene-TAMRA and Internal control-HEX channels are used. *Fluorescence measured at 60° C.			

Note: Please select "None" in both Passive reference and Quencher.

c. Interpretation of Results:

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. ≤34 Ct of unknown samples should be considered for result interpretation. The Lower detection limit (LoD) of AstraArt FLU/RSV/COVID-19 qPCR Kit is defined as 15.6 copies/reaction.

Control	FLU (TxRd)	RSV (Cy5)	COVID-19 (FAM)	Conclusion
***Positive Control**	+	+	+	Valid
***Negative Control**	-	-	-	Valid

Table 3: Conclusion:

Sample type	FLU (TxRd)	RSV (Cy5)	N gene (FAM)	E gene (TAMRA)	IC (HEX)	Conclusion
Sample	-	-	+	+	+	COVID-19 Positive
Sample	+	-	-	-	+	FLU Positive
Sample	-	+	-	-	+	RSV Positive
Sample	+	+	-	-	+	FLU and RSV Positive
Sample	+	-	+	+	+/-	FLU and COVID-19 Positive
Sample	-	+	+	+	+/-	RSV and COVID-19 Positive
Sample	+	+	+	+	+/-	FLU, RSV, COVID-19 positive
Sample	-	-	-	-	+	Negative
Sample	-	-	-	-	-	Possible inhibition of PCR/No sample collected

LIMITATION OF THE PROCEDURE:

1. The use of this assay as an in vitro diagnostic and is limited to laboratories that are certified to perform high complexity tests.
2. This kit is used for qualitative detection of FLU, RSV and COVID-19 RNA from specimens. The results do not reflect the viral load in the original specimen.
3. 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.
4. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure.
5. Amplification and detection of the Kit 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:

 Date of manufacture  Contains sufficient for < n >  Use-by-date

 Do not use if package is damaged  Manufacturer  Batch Code

 CE mark of Conformity  Refer to the instructions  ISO  GMP

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