# Aridia® CoVid-19 Real Time PCR Detection Kit

**(For Qualitative Detection)**

**REF AP0010**

IVD -25°C -15°C



## APPLICATION

The *Aridia*® CoVid-19 Real Time PCR Detection Kit is an in vitro diagnostic test, based on real-time PCR technology, for the qualitative detection of coronavirus specific RNA. Coronavirus belongs to the family of Coronaviridae, in the order of Nidovirales. It is formed by a positive-sense single-stranded RNA, usually appears spherical with a size of 80-120nm and with crown-like spikes on the surface. This large family of virus is commonly circulating among vertebrates, such as camels, cats and bats. Novel coronavirus (COVID-19) has been identified as a new strain of coronavirus. It can cause viral pneumonia and dyspnea in humans.

## KIT COMPONENTS

| S.No | Component | AP0010-96 Volume [µL/Vial] |
|---|---|---|
| 1 | Master A | 85 |
| 2 | Master B | 200 |
| 3 | Buffer | 400 |
| 4 | Primer - Probe Mix | 300 |
| 5 | Positive Control for N Gene | 100 |
| 6 | Positive Control for ORF1ab | 100 |
| 7 | Water (PCR grade) | 2000 |

## STORAGE

The *Aridia*® CoVid-19 Real Time PCR Detection Kit is shipped with dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact Athenese-Dx Pvt. Ltd. for assistance. All components should be stored between -25°C and -15°C upon arrival.

Repeated thawing and freezing of Master reagents (more than three time) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently. Storage between +2°C and +8°C should not exceed a period of four hours. Protect Primer Mix from light.

**Product Information**

## INTENDED USE

*Aridia*® CoVid-19 Real Time PCR Detection Kit is designed for the specific identification and differentiation of 2019 Novel Coronavirus (SARS-CoV-2) in respiratory samples from patients with signs and symptoms of COVID-19 infection. This test is intended for use as an aid in the diagnosis of SARS-CoV-2 in combination with clinical and epidemiological risk factors. RNA is extracted from respiratory specimens, amplified using RT-PCR and detected using fluorescent reporter dye probes specific for SARS-CoV-2.

## PRINCIPLE OF THE TEST

*Aridia*® CoVid-19 Real Time PCR Detection Kit is designed for the diagnosis of SARS-CoV-2 in respiratory samples. The detection is done in one step real time Reverse Transcriptase format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by the amplification of a conserved region of ORF1ab and N genes for SARS-CoV-2 using specific primers and a fluorescent-labeled probe.

*Aridia*® CoVid-19 Real Time PCR Detection Kit is based on the 5´-exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bounded to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of target template. This fluorescence can be measured on Real Time PCR platforms.

*Aridia*® CoVid-19 Real Time PCR Detection Kit contains all the components necessary for real time PCR assay (specific primers/probes, and Master Mix that includes Enzymes, dNTPs, $MgCl_2$ etc.) in a stabilized format, as well as an internal control to monitor PCR inhibition. N gene is amplified and detected in FAM channel, ORF1ab gene is amplified and detected in HEX channel and the internal control (IC) in ROX channel.

**Sample Volume** : 5 µL (extracted RNA specimen) .
**Test Time** : ~1.5 hours (dependent on the real-time Thermocyclers used).
**Storage** : The kit is to be stored at temperature of -20 ºC.
**Shelf Life** : Targeted shelf life is ~ 12 months from the date of manufacture.
**Safety Information** : This product utilizes no biohazardous materials, radioactive materials, narcotic reagents or their derivatives, viruses or their reagents, nor any metabolic by-products

## REAL-TIME PCR INSTRUMENTS

The *Aridia*® CoVid-19 Real Time PCR Detection Kit can be used with the following real-time PCR instruments:
ALTA RT-96 (Athenese-Dx Pvt. Ltd.)
ALTA RT-48 (Athenese-Dx Pvt. Ltd.)
ABI Prism® 7500 SDS (Applied Biosystems)
Rotor-Gene® Q5/6 plex Platform (QIAGEN)
CFX96™ Real-Time PCR Detection System (Bio-Rad)
LightCycler® 480 Instrument II (Roche)

**Note:**

Please ensure that all instruments are properly installed, calibrated, checked and maintained according to the manufacturer instructions and recommendations, before using the *Aridia*® CoVid-19 Real Time PCR Detection Kit to test the specimen.

## PROCEDURE

**Sample Preparation**

Extracted RNA is the starting material for the *Aridia*® CoVid-19 Real Time PCR Detection Kit The quality of the extracted RNA has a profound impact on the performance of the entire test system. It is suggested to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

QIAamp® Viral RNA Mini Kit (QIAGEN)

QIAsymphony® (QIAGEN)

MagNA Pure 96 System (Roche)

Maxwell® 16 IVD Instrument (Promega)

*Aridia*® Viral DNA/RNA Extraction Kit (Athenese-Dx Pvt. Ltd.)

*Aridia*® DNA/RNA Complete Extraction Kit (Athenese-Dx Pvt. Ltd.)

Alternative nucleic acid extraction systems and kits might also be appropriate.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 13,000 rpm, using a new collection tube, prior to the elution of the nucleic acid.

## ASSAY PROCEDURE

**Positive Control Preparation**

The Positive Control (PC) contains high copy number templates. Contamination of the PCR environment, equipment, and/or kit components with the Positive Control can lead to false positive results. Thus, it should be opened and handled in a separate laboratory area, away from the PCR amplification area and other kit components. To ensure complete rehydration, vortex the tube thoroughly and centrifuge briefly. Store aliquots at -20 ºC.

## PCR PROTOCOL

**Master Mix Setup**

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The *Aridia*® CoVid-19 Real Time PCR Detection Kit contains a human RNAse P gene as Internal Control (IC). The Internal RNAse P control provides a nucleic acid extraction procedural control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

| Component | Volume/Reaction |
|---|---|
| Master A | 0.8 µL |
| Master B | 2.0 µl |
| Buffer | 4.0 µL |
| Primer - Probe Mix | 3.0 µL |
| Molecular Biology Grade Water(MBGW) | 5.2 µL |
| Master Mix Total | 15.0 µL |
| Positive Control/Sample RNA/Negative Control | 5 µL |
| **Total** | **20 µL** |

**Program your real-time PCR Thermocyclers**

Calculate the number of required reactions including samples and controls (at least one Positive Control reaction and one Negative Control reaction must be included with each run). Program your thermocycler to the following conditions below:

| Cycles | Step | Time | Temperature |
|---|---|---|---|
| 1 | RT incubation | 15 minutes | 50 ºC |
| 1 | Initial denaturation | 2 min 30 seconds | 95 ºC |
| 40 | Denaturation | 15 seconds | 94 ºC |
| | Annealing (Data collection*) | 40 seconds | 55 ºC |

Set the fluorescence data collection during the extension step (*) through the **FAM (N-Gene), HEX (ORF1ab), and ROX channel (Internal Control).**

**Starting the real-time PCR run**

Place the strips/plate into the real-time PCR thermocycler. Ensure that the configuration/order of the samples and control wells matches the real-time PCR experimental plate setup in the software. Start the run.

**Data Analysis**

Please refer to the user manual of the respective instrument for basic information regarding data analysis on specific real-time PCR instruments.

For detailed instructions regarding the analysis of the data generated with the *Aridia*® CoVid-19 Real Time PCR Detection Kit on different real-time PCR instruments, please contact our Technical Support (see chapter 8. Technical Assistance).

## INTERPRETATION OF RESULTS

**Qualitative Analysis**

| Detection Channel | | | Result Interpretation |
|---|---|---|---|
| FAM | HEX | ROX | |
| + | + | +* | Target ORF1ab gene and target N gene specific RNA detected.<br><br>Internal Control Positive |
| + | - | +* | Target N gene specific RNA detected.<br><br>Internal Control Positive |
| - | + | +* | Target ORF1ab gene specific RNA detected.<br><br>Internal Control Positive |
| - | - | + | Neither target ORF1ab gene nor target N Gene specific RNA detected.<br><br>Internal Control Positive |
| - | - | - | RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample. |

* Detection of the Internal Control in the ROX detection channel is not required for positive results either in the FAM detection channel or in the HEX detection channel. A high positive RNA load in the sample can lead to reduced or absent Internal Control signals. (Absence of Internal control signal in the ROX channel for high positive clinical samples may be observed and the test result is considered positive.)

## READING THE GRAPH

**Step-1**

**Internal control**

- Select the test sample wells only and observe the internal control amplification graph under ROX channel. A successful amplification of the internal control must have a Ct value less that 35 indicating that the samples are purified well and there is no PCR inhibition in the reaction. Any samples that have values beyond 35 for internal control indicates that the sample has some issues in the purification or the presence of some inhibitors in the PCR reaction.
- An exceptional case is that when the Ct value for internal control is greater than 35 for a sample may be accepted when the test sample is high positive for COVID-19. Any test sample whose internal control Ct value is greater than 35 must be purified again and the PCR must be run again for confirmation.

**Step-2**

**No Template control and Positive control**

- Select the NTC and Positive control, select FAM channel & HEX channel, and view the graph for amplification.
- The NTC must be flat with no Ct value. If required, adjust the threshold value just above the NTC.
- The PC or Standards must be amplified.
- NTC justifies NO contamination in the reagent as well as fine pipetting and its environment.
- PC justifies the reagents storage conditions and reaction parameters are as prescribed.

**Step-3**

**Test samples**

- In FAM & HEX channel, Select the NTC well and any one of the test sample well, analyze the graph/amplification. Every time, select the NTC well and the test sample well, one by one analyze. Any test sample whose Ct value is beyond 40 must be re-purified and rerun the assay.

## Clinical performance

A Total of 160 specimens tested by the Aridia® CoVid-19 Real Time PCR Detection Kit. The results are in the following table.

| | Aridia® CoVid-19 Real Time PCR Detection Kit | | |
|---|---|---|---|
| RT-PCR characterized specimens | Positive | Negative | Total |
| Positive | 74 | 1 | 75 |
| Negative | 0 | 85 | 85 |
| Total | 74 | 86 | 160 |

Sensitivity: 98.7%, Specificity: 100%.

**Index of symbols**

| | | |
|---|---|---|
| Consult instructions for use | Batch code | Tests per kit |
| For *in vitro* diagnostic use only | Do not re-use | Keep dry |
| Temperature limit - 25 °C to - 15 °C | Date of manufacture | Biologial Risks |
| Manufacturer | Use-by date | |

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