iAMP® COVID-19 Detection Kit

(For Research Use Only; Not For Use In Diagnostic Procedure)

REF **iAMP-COVID19-100-RUO**

Instructions For Use

V1.0

March, 2020

Σ 100

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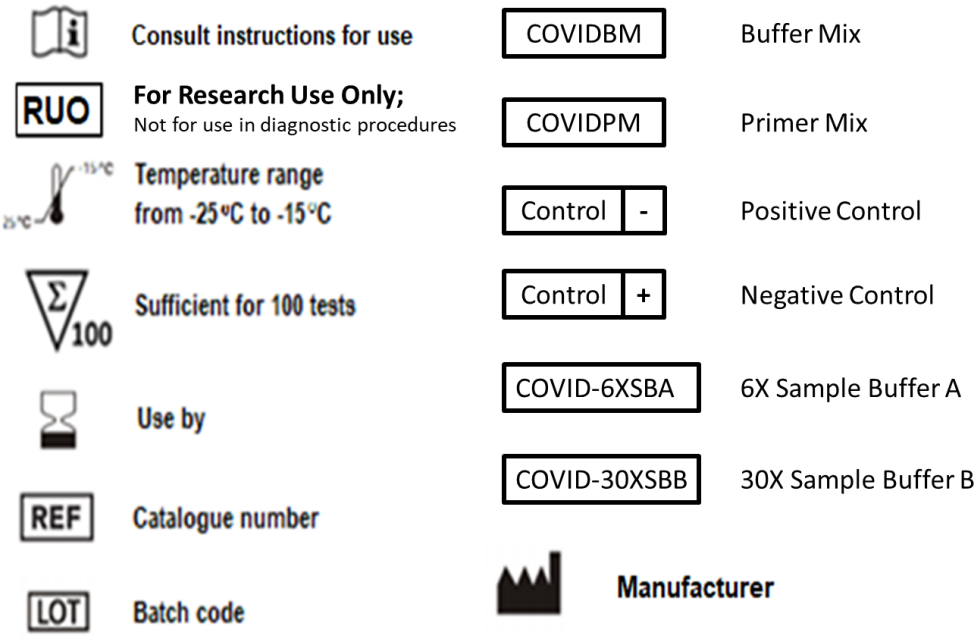
IMPORTANT NOTICE

The instruction for use must be read carefully prior to use and followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from these instructions.

SYMBOLS



INTENDED USE

iAMP COVID-19 Detection Kit is a multiplexed, real-time fluorescent RT-isothermal assay based on Atila's proprietary isothermal amplification technology intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs from individuals with signs and symptoms of infection who are suspected of COVID-19. Testing is limited to research use only.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal/oropharyngeal swabs during the acute phase of infection. Positive results are indicative of active infection.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for decisions on infection status. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The iAMP COVID-19 Detection Kit is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification and laboratory research procedures. The iAMP COVID-19 Detection Kit is only for research use.

SUMMARY AND EXPLANATION OF THE TEST

Test Overview

The iAMP COVID-19 Detection Kit is a real-time reverse transcription isothermal amplification test. The test is based on a proprietary isothermal amplification technology termed OMEGA amplification (Patent: WO 2017/205510 A1). OMEGA primer sets are designed to specifically detect RNA and later cDNA from N gene and ORF-1ab of the SARS-CoV-2 virus in nasopharyngeal/oropharyngeal swabs from patients with signs and symptoms of infection who are suspected of COVID-19.

Test Principle

The iAMP COVID-19 Detection Kit is intended to detect COVID-19 **directly from raw samples without RNA extraction process. Swab specimens in 1X Sample Buffer Mix with a 15-min incubation at room temperature can be directly used for OMEGA isothermal amplification and signal detection.**

After sample processing, both reverse transcription and nucleic acid amplification take place at 61°C. Target sequence in the specimens is amplified with N/ORF-1ab primer sets that are specific to SARS-CoV-2. During the amplification, fluorescence resonance energy transfer (FRET) probes can be incorporated in the amplification products. Upon the incorporation, fluorescence is generated and can be monitored by fluorescence reader in a real time fashion.

KIT COMPONENTS

1.	Primer Mix (COVIDPM)	540 μL X 1 tube
2.	Buffer Mix (COVIDBM)	540 μL X 1 tube
3.	Positive Control Template (COVIDPC)	300 μL X 1 tube
4.	Negative Control Template (COVIDNC)	300 μL X 1 tube
5.	Instructions for use	1 booklet
6.	6X iAMP COVID-19 Sample Buffer A (COVID-SBA)	1.2 mL X 5 tubes
7.	30X iAMP COVID-19 Sample Buffer B (COVID-30XSBB)	0.24mL X 5 tubes

EQUIPMENTS & MATERIALS REQUIRED BUT NOT SUPPLIED with the kit

1. iAMP COVID-19 Sample Collection Device (iAMP-COVID19-SCD, Including: synthetic fiber swabs with plastic shafts, and 1.5mL collection tubes) ; 100 units needed for one iAMP COVID-19 Detection Kit
2. Water: nuclease-free H_2O
3. Surface decontaminants
4. Real-time PCR system with **FAM/HEX** fluorescence channels (Atila Powergene 9600 Plus Real-Time System, Biorad CFX96 Real-Time System or other compatible instruments).
5. Adjustable pipettes with corresponding filter-plugged pipette tips
6. Disposable powder-free gloves and other personal protective equipment

7. Vortex mixer or equivalent
8. PCR tubes/strips with caps or plate film
9. PCR tube/plate holder
10. 1.5 mL and 2 mL microcentrifuge tubes and racks
11. Bench top centrifuge

KIT STORAGE INFORMATION

All kit reagents **(COVIDBM, COVIDPM, COVIDNC, COVIDPC, COVID-6XSBA, and COVID-30XSBB)** should be stored at -20°C freezer for long time storage. Shelf life and open kit stability is not available yet. Presumably the shelf-life of the kit is 1 year and the open kit stability is 6 months when kit is properly stored.

SPECIMENS

Biosafety Precautions

Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens. Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines. For more information, refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation for COVID-19.

Acceptable Specimens

- Direct nasopharyngeal or oropharyngeal dry swabs collected with Atila Sample Collection tube. Alternatively, synthetic fiber swabs with plastic shafts and 1.5mL collection tubes from other vendors can be used.

Specimen Handling and Storage

- Use freshly collected specimens for optimal test performance.
- Specimens can be stored at room temperature for up to 12 hours or at 4ºC for up to 48 hours after collection and before sample processing.
- If a delay in sample processing is expected, store dry swab specimens at -70ºC or lower. Avoid freeze-thaw cycles of the specimens.
- **Processed specimens need to be tested within 2 hours. If a delay in sample testing is expected, store processed specimens at 4 ºC for up to 12 hours.**

Specimen Rejection criteria

- Specimens not kept as instructed.
- Incomplete specimen labeling or documentation.
- Inappropriate specimen type.
- Insufficient specimen volume.

QUALITY CONTROL

Due to the sensitivity of iAMP COVID-19 reaction, these assays should be conducted using strict quality control and quality assurance procedures. Following these guidelines will help minimize chance of false-positive or false-negative amplification.

General Considerations

- Personnel must be familiar with the protocol and equipments/instruments used.
- Maintain separate areas and dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips, gowns and gloves) for assay reagent setup and handling of processed samples.
- Workflow must always be from the clean area to the dirty area.
- Wear clean disposable gowns and new, previously unworn, powder-free gloves during assay reagent setup and handling of processed samples. Change gloves whenever contamination is suspected.
- Store primer/probes and enzyme master mix at appropriate temperatures (see package inserts). Do not use reagents beyond their expiry dates.
- Keep reagent tubes and reactions capped as much as possible.
- Clean and decontaminate surfaces.
- Do not bring processed samples or reaction products into the assay setup area.
- Use aerosol barrier (filter) pipette tips only.

Assay Controls

Assay controls should be tested concurrently with all test samples in each instrumental run.

- PC - positive template control with an expected Ct value range, serves as a control for amplification and detection of SARS-CoV-2 RNA.
- NC - negative template control, serves to verify that analyte contamination does not occur during reaction setup.

TEST PROCEDURE

Specimen Processing

1. From the kit, take 6X iAMP COVID-19 Sample Buffer A (COVID-6XSBA) and 30X iAMP COVID-19 Sample Buffer B (COVID-30XSBB) out. Each tube contains enough volume to process 20 dry swabs. Only thaw the number of COVID-SBA tubes that will be enough for each round of sample processing.
2. Make **1X Sample Buffer Mix** by mixing **(60 x N) µL** 6X iAMP COVID-19 Sample Buffer A and **(12 X N) µL** 30X iAMP COVID-19 Sample Buffer B with **(288 x N) µL** nuclease-free H2O (N represents the number of dry swab specimens to process). Vortex briefly.
3. Load **350 µL** prepared 1X Sample Buffer Mix into each sample tube. Seal the tube cap securely and vortex briefly.
4. Place the sample tube on the bench for 15 min.

Reaction assembly and run:

1. From the kit, take N+2 PCR tubes (N represents number of specimen samples to be tested). Then make **reaction master mix** by adding [**5.2** X (N+2)]µL **PM** (yellow cap) and [**5.2** X (N+2)] µL **BM** (red cap) in a 1.5 mL centrifuge tube, gently vortex and spin, and add **10 µL** reaction master mix to the bottom of each of the PCR tubes.

2. Briefly spin the tubes to bring down the liquid to the bottom of the sample tubes. Add **15 µL** of processed specimen samples (Sample #1 to #N) from step of **"Specimen Processing"** to corresponding reaction PCR tubes from above **Step 1**. For negative control, add 15 µL of Negative Control Template (blue cap) into reaction tube #(N+1). For positive control, add 15 µL of Positive Control Template (pink cap) into reaction tube #(N+2).

3. Cap all the tubes securely. Gently vortex the tubes to mix all the reagents.
4. Briefly spin the tubes in a centrifuge to bring down all the liquid to the bottom of the wells.
5. Set the reaction condition using a compatible real-time PCR machine (All real-time PCR instruments capable of measuring fluorescence in FAM/HEX channel in real-time. Such instruments include but not limited to: Atila Powergene 9600 Plus Real-Time System, Biorad CFX96 Real-Time PCR Detection System, Roche LightCycler 480 Real-Time PCR System, Applied Biosystems 7500 Real-Time PCR System, etc.)

Open a new PCR program and do the following edits:

1) For denaturation step: set 61˚C for 30 seconds
2) For signal amplification step: run 50 cycles, set 61˚C for 1 min for each cycle, while fluorescence reading was taken at the **FAM/HEX** channels at the end of each cycle.

6. Put the reaction tubes into the sample holder and bring into the real-time PCR machine, close the lid, and start the reaction run.

After the run, take out the sample plate and discard them immediately**.** To avoid contamination **DO NOT OPEN THE REACTION TUBE AFTER THE REACTION**.

AMPLIFICATION RESULT INTERPRETATION

iAMP COVID-19 Detection Kit Controls – Positive, Negative and Internal Controls

- Quality control of a test- every instrument run should include:
  a) **Negative Control Template**- serves to verify that analyte contamination does not occur during reaction setup. There should be NO exponential amplification curve shown in any channel in negative control template, otherwise the test is invalid.
  b) **Positive Control Template**- serves as a control for amplification and detection of SARS-CoV-2 RNA (ORF1ab and/or N). It should show exponential curves in both channels, and Ct in each channel should be less than 30, otherwise the test is invalid.

  Quality control of an instrument run should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

- **Internal Control in each specimen**- also serves as a nucleic acid extraction procedural control that validates both the sufficiency of sample collection as well as nucleic extraction procedure and reagent integrity. Internal control is measured in HEX channel in this assay kit. If a sample shows no exponential amplification curve in HEX channel but an exponential curve in FAM channel, the sample is still reported as a valid run and will be interpreted following instructions as below. If there is no exponential amplification curve in any channel in a sample, the sample test result is invalid, and a new sample of the patient needs to be collected, processed and re-tested.

Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the specimen results cannot be interpreted.

- **Sample test result interpretation**- an exponential amplification curve showing up at any of

the two channels (**FAM/HEX)** indicates the presence of corresponding assayed analyte as indicated below**:**

	Analyte
FAM Channel	ORF1ab and/or N
HEX Channel	Sample internal control

A summary of sample test result interpretation is shown as below.

	FAM	**HEX**	**Result**
Case A	-	-	Invalid*
Case B	-	+	SARS-CoV-2 not detected
Case C	+	+/-	SARS-CoV-2 positive

*: Repeat test. If result remains invalid, re-sampling is needed

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