2019

CO-DIAGNOSTICS INC.

# Logix Smart Coronavirus Disease 2019 (COVID-19) Kit

REF **COVID-K-001**

Logix Smart Coronavirus Disease 2019 (COVID-19) Test Kit

CO-DIAGNOSTICS, INC.

RUO

CO-DIAGNOSTICS, INC. | 2401 Foothill Dr., Ste D, Salt Lake City, UT 84109 USA

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RUO

## 1 INTENDED USE

The **Logix Smart Coronavirus Disease 2019 (COVID-19)** kit is a research use only test, based on real-time PCR (qPCR) technology, for the qualitative detection of the Coronavirus Disease 2019 (COVID-19).

**For research use only (RUO). Not for use in diagnostics procedures.**

## 2 KIT COMPONENTS

| Lid Color | Component | Symbol | Catalog Number | Description | Amount |
|---|---|---|---|---|---|
| **Black** | Logix Smart™ COVID Master Mix | MM | COVID-MM-001 | Proprietary blend of CoPrimers™ and PCR reagents | 1x500µL (100 reactions) |
| **Red** | Logix Smart™ COVID Positive Control | PC | COVID-PC-001 | Proprietary blend of target templates | 1x500µL (100 reactions) |
| **Clear** | Nuclease-Free Water | NTC | GEN-NF-001 | DNase/RNase-free water | 1x500µL (100 reactions) |

- Kit Catalog Number is COVID-K- 001. Contact Sales at 801-438-1036 ext. 02 or at www.codiagnostics.com/contact/ to order.

## 3 LOGIX SMART™ COVID-19 STORAGE, HANDLING, & DISPOSAL

- The **Logix Smart™ Coronavirus Disease 2019 (COVID-19)** kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more of the components are not frozen upon receipt, or are compromised during shipment, contact your distributor for assistance.
  - Upon receipt of kit, laboratory should follow internal procedures for quality control.
- All components should be stored below -20°C upon arrival to prevent degradation of reagents.
- Repeated thawing and freezing of components (more than four times) should be avoided, specifically the master mix, as this might affect the performance of the assay. The reagents should be frozen in multiple aliquots if they are to be used intermittently.
- Co-Diagnostics recommends storage between +2°C and +8°C should not exceed a period of 4 hours.

- If you work in an area prone to power outages, it is recommended to have a back-up generator for your freezer as well as a temperature data log system to ensure that the **Logix Smart™ COVID-19** test kit remains frozen at -20°C.
- Protect **Master Mix** from light.
- Expired products should not be used, as the integrity of the components cannot be guaranteed.
- The product is not a biological waste. See Safety Data Sheets (SDS) for hazard classification. Disposal should be in accordance with applicable regional, national, and local laws and regulations.

## 4 WARNINGS AND PRECAUTIONS

**WARNING!** 

Read this *Instructions for Use* carefully before using the product. Before first use check the components for:

- Integrity
- Frozenness upon arrival

Users should pay attention to the following:

- Use of this product should be limited to personnel instructed and trained in the techniques of real-time PCR.
- Samples should always be treated as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, lab coat, and eye protection when handling samples. Always wear gloves when handling kit components.
- Always use DNase/RNase-free disposable pipette tips with filters.
- Use segregated working areas for sample preparation, reaction setup, and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional workflow. To prevent contamination, change PPE between areas.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents. Dedicate supplies and equipment to separate working areas and do not move them from one area to another.
- Consult appropriate Safety data Sheets (SDS) for safety. The SDS for the **Logix Smart™ COVID-19** test kit is provided with the shipment. If not provided with shipment the SDS can be retrieved from Co-Diagnostics website at the link: http://codiagnostics.com/resources/safety-data-sheets/
- Do not open the reaction tubes/plates post amplification.
- Do not autoclave reaction tubes/plates after the PCR, since this will not degrade the amplified nucleic acid and will pose a risk to the laboratory area to contamination.
- Do not use components of the kit that have passed expiration date.
- Discard sample and assay waste according to your local safety regulations.

# 5 BACKGROUND INFORMATION

## 5.1 Coronavirus Disease 2019 (COVID-19)

- **About:** Coronavirus Disease 2019 is a contagious, zoonotic virus that causes respiratory infection varying from common cold symptoms to severe pneumonia. Much of the 2019 Novel coronavirus is unknown and the current knowledge is drawn from what is known about similar coronaviruses. Coronaviruses are a large family of viruses that are common in many different species of animals, including camels, cattle, cats, and bats. Rarely, animal coronaviruses can infect people and then spread throughout the population, which was seen with MERS, SARS, and now with the COVID-19 strain.
- **The virus:** A positive-sense, single-stranded RNA virus, from the Coronaviridae family, genus Betacoronavirus. Comparisons of genetic sequences between the 2019 novel and other coronaviruses have shown that the COVID-19 has similarities to SARS-CoV (79.5%).
- **Signs and Symptoms:** Reported symptoms include fever, fatigue, dry cough, shortness of breath, and respiratory distress. Cases of severe infection can result in pneumonia, and kidney failure. Based on early evidence, many of those who died had other conditions such as hypertension, diabetes, or cardiovascular disease that impaired their immune systems.
- **Transmission:** The virus has spread with human-to-human transmission, which has been shown among close contacts (about 6 feet) via respiratory droplets. They are produced when an infected person coughs or sneezes, like other respiratory infections, such as influenza or the common cold. It's not clear if COVID-19 strain can be transmitted through indirect contact via contaminated surfaces or objects. Typically, viruses seem to be more contagious when people are most symptomatic (the sickest). The incubation period may vary a lot from person to person. (CDC, 2020).

# 6 PRODUCT DESCRIPTION

The **Logix Smart™ Coronavirus Disease 2019 (COVID-19)** kit is a research use only test, based on real-time polymerase chain reaction technology. It tests for the presence or absence of ribonucleic acid (RNA) of the Coronavirus Disease 2019 (COVID-19) microorganism.
The **Logix Smart™ COVID-19** test includes an internal control to identify possible qPCR inhibition, confirm the integrity of the reagents, and verify the quality of sample extraction. The **Logix Smart™ COVID-19** test also includes a positive control which includes a synthetic RNA molecule carrying sequences that are homologous to Coronavirus Disease 2019 (COVID-19) microorganism and are targeted by this assay. Positive controls represent a source of amplicon contamination. Precautions should be taken to prevent and minimize the risk.

CoPrimers™ included in the **Logix Smart™ COVID-19** test are:

- CoPrimers™ that target COVID-19 and are labelled with the FAM™ fluorophore
- CoPrimers™ that target the Internal Positive Control (IPC) DNA are labelled with CAL Fluor® Red 610 fluorophore

# 7 MATERIALS AND DEVICES (REQUIRED BUT NOT PROVIDED)

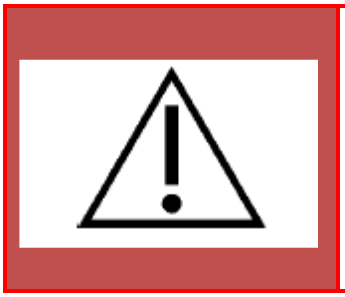
- Appropriate 2-channel real-time PCR instrument, compatible with the fluorophores used in this test.
- Appropriate nucleic acid extraction system or kit
- Vortex mixer
- Centrifuge with a rotor for 2 mL microcentrifuge tubes
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Ice
- Class II Biological Safety Cabinet (BSC), ideally in a BSL-2 facility

All instruments should be properly installed, calibrated, and maintained according to the manufacturer's instructions and recommendations. Do **not** use instruments with outdated calibration.

# 8 PROCEDURE

## 8.1 Sample Preparation

The quality of the RNA from the sample extraction is essential for the performance of **Logix Smart™ COVID-19**. The extraction protocol should be performed following manufacturer's instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with **Logix Smart™ COVID-19** must be validated by the user.



If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

The use of carrier RNA can be crucial for extraction efficiency and the stability of the extracted nucleic acid.

## 8.2 Logix Smart™ Coronavirus Disease 2019 (COVID-19) Reagent Setup

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by 70% ethanol, or another equivalent method of cleaning that disinfects and degrades nucleic acids.
- All **Logix Smart™ COVID-19** Master Mix, Positive Control (PC), no template control nuclease-free water (NTC), and sample tubes should be vortexed for 3 seconds, and briefly spun down before using to ensure properly mixed reagents, and to remove any condensation or residue from the lids.

- Thaw all reagents and samples on **ice**, or on a cold block, before starting setup.

## 8.3 Reaction Setup

8.3.1 Every reaction setup should include enough reaction wells for the number of samples and at least one positive control (PC) and one no template control (NTC) (**# samples + 2 = total reaction wells needed**). Example: 5 samples to test + 1 PC well + 1 NTC well = 7 total reaction wells.

8.3.2 All pipetting should be done on **ice,** if possible. Pipetting of samples and PCs is recommended to be done in a separate area from master mix and no template control if possible, or at a separate time, in order to prevent possible contamination of reagents. Change pipette tips in-between samples and change pipette tips after pipetting each component. Pipet PC last if possible, to avoid contamination events.

8.3.3 Pipet 5 µL of **Master Mix** into each well being used in an appropriate optical plate or optical reaction tube (example: CoDx Box real-time PCR instrument uses 48-well reaction tubes).

8.3.4 Pipet 5 µL of sample (elution from nucleic acid extraction) or 5 µL of a control (**NTC** or **Positive Control**) to the appropriate well(s). At least one positive and one NTC control must be included in each run.

8.3.5 Seal the reaction plate with an optical adhesive film or the reaction tubes with appropriate lids.

8.3.6 Place plate or tubes into real-time PCR instrument in the correct orientation and start run.

## 8.4 PCR Instrument Setup

8.4.1 For basic information regarding setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For programming instructions questions regarding the use of other real-time PCR instruments please contact the Laboratory 801-438-1036 ext.04 or at www.codiagnostics.com/contact/.

8.4.2 If using a Co-Diagnostics Inc. CoDx Box, contact the Laboratory 801-438-1036 ext. 04 or at www.codiagnostics.com/contact/ for the template file for download. The template file comes pre-programmed with the PCR instrument setup described in this section. When not using a template, or using another device, use the settings outlined below to program the PCR instrument.

8.4.2.1 In order to achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined below.

8.4.3 Define the following settings:

| | |
|---|---|
| Reaction Volume | 10 µL |
| Ramp Rate | Default |
| Passive Reference | None |

8.4.4 Program PCR instrument with the cycling conditions below:

| | Stage | Cycles | Temperature | Time |
|---|---|---|---|---|
| Reverse Transcription | Activation | 1 | 45˚C | 15 minutes |
| Initial Denaturation | Hold | 1 | 95˚C | 2 minutes |
| Amplification | Cycling | 50 | 95˚C | 3 seconds |
| | | | 55˚C | 32 seconds |

8.4.1 Ensure that the PCR instrument being used is compatible with the fluorophores listed below. Some devices may not have options for the quencher. If needing help or you have questions, contact Co-Diagnostics Inc. technical support at 801-438-1036 ext. 04 or at: www.codiagnostics.com/contact/.

8.4.2 Define the fluorescence detectors (dyes):

| Target | Detector Name | Reporter | Quencher |
|---|---|---|---|
| COVID-19 specific RNA | COVID-19 | FAM™ | BHQ® - 1 |
| RNaseP specific DNA (IPC) | RNaseP | CAL Flour® Red 610 | BHQ® - 2 |

- When the run is finished, ensure that the run file is saved.

## 9 DATA ANALYSIS

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

### 9.1 Validity of Test Runs

9.1.1 Valid Test Run

- Check to see that both the positive and no template control passed.

9.1.1.1 The following control conditions must be met:

| Control Type | Control Name | Purpose of Control | COVID-19 FAM channel | Internal Control (RNaseP) CF610 channel |
|---|---|---|---|---|
| **COVID Positive Control** | COVID-19 (FAM™) | Verifies the performance of the master mix | + | + |
| | RNaseP (CF®610) | | | |
| **No Template Control** | Master Mix + Water | Verifies the reagents are free of contamination | - | - |

- If controls pass, interpret the sample results.

9.1.2 Invalid Test Run

9.1.2.1 If any of the controls fail, the run is invalid. Document the run and initiate troubleshooting.

### 9.2 Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes:

- Positive
- Negative
- Inconclusive

A **Positive** result will show an amplification curve or cycle threshold value for COVID-19. The cut off value should be determined by in house validation testing. However, internal studies have shown rare primer-dimer formation or other non-specific amplification at 45 cycles. This fact can be attributed to the nature of the CoPrimers™ (Satterfield, 2014) (Poritz & Ririe, 2014). The amplification of the RNaseP (IPC) shows that the extraction was successful.

A **Negative** result will show no amplification for COVID-19; The absence of a curve for COVID-19 strain indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

An **Inconclusive** result will occur if any of the controls fail. See troubleshooting.

The interpretation of results can be translated to the following table (version KM):

| | Sample Result | | Logix Smart™ COVID-19 Positive Control | No Template Control (NTC) (Master Mix + Water) | Interpretation |
|---|---|---|---|---|---|
| | COVID-19 FAM channel | Internal Positive Control (RNaseP) CF610 channel | | | |
| Instrument Reading | + | + | + | - | COVID-19 + |
| | - | + | + | - | COVID-19 - |
| | Any Result ( +/- ) | - | + | - | Inconclusive: See Troubleshooting |
| | | + | - | - | |
| | | + | + | + | |

**The cut off value will determine what results are to be considered positive or negative. It should be determined by the assay development. Red boxes indicate an undesired result, while Green boxes indicate an expected result.**

**A result will be considered as positive (+) if a Cq value of ≤45 is determined, while any values ≥45 should be considered as negative (-).**

## 10 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and we would like to be informed of any issues with the **Logix Smart™ COVID-19** test kit, even if the recommended steps for troubleshooting solve the issue. To give feedback please fill out the Customer Feedback Form by visiting www.codiagnostics.com/contact/feedback/

### 10.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Presently, the expiration date of this product has been established as 12 months. It is not recommended to use expired kit reagents, doing so may lead to inaccurate results.

Always use the most recent version of this document for updates as more stability information will be added when studies are completed.

## 10.2 User Errors

Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel.

It is essential for the user to have some molecular biology experience and be familiar with proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection. Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment, such as pipettes and real-time PCR instruments, should be calibrated when applicable.

90 minutes of online training for Good Laboratory Practices for Molecular Genetics Testing (Centers for Disease Control and Prevention, 2017) is available at the CDC website at the following link https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html

## 10.3 Invalid Results/Inconclusive Results

### 10.3.1 **Logix Smart™ COVID-19 Positive Control** not amplifying

No amplification from the PC could be the result of one or multiple factors, such as:

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,
- **Logix Smart™ COVID-19 Master Mix** or **Logix Smart™ COVID-19 Positive Control** degradation (result of reagents being at temperatures above -20°C for an extended period),
- Use of expired reagents,
- or the wrong reagents being used.

Without further evidence, it is best to disregard the results from the samples and re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error, and the test should be reprocessed from extraction, depending on the investigation results and risks identified in the process. If failure of the positive control happens a third time after re-extraction and re-amplification, open a new **Logix Smart™ COVID-19 Positive Control** or **Master Mix**, and retest. If still failing, please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 visiting www.codiagnostics.com/contact/.

### 10.3.2 **Internal Positive Control (IPC)** not amplifying in samples

No amplification from the RNaseP channel could be the result of one or multiple factors, such as:

- Not enough nuclear material in the sample,
- PCR inhibitors such as: ethanol and/or heparin,
- the extraction was performed incorrectly,
- or the extraction kit used is not compatible or has a step that eliminates a crucial element for extraction.
- Sample origin is not compatible to the test intended use.
- Note: Positive amplification in the COVID-19 channel indicates a positive result despite the lack of concurrent amplification in the IPC channel. The IPC in Co-Diagnostics test kits may vary due to the test application. In most cases, amplification is dependent on the presence of human genomic DNA (gDNA) in the extracted sample, the amount of which is governed by the type of

the sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g. CSF, urine, cell lysates, etc.) may not contain the human RNaseP gene. In such cases, the two negative markers indicate a true negative result for Coronavirus Disease 2019 (COVID-19).

Negative results cannot be trusted and re-testing by re-amplification should be performed. If the IPC fails again, then samples should be re-extracted and re-amplified. If it fails after a third time an investigation should be conducted to identify possible causes for error. If the cause for the error is clear, the test can either be called as **inconclusive** due to either PCR inhibitors being present or not enough nuclear material being present. If the cause for error is unclear, contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or by visiting www.codiagnostics.com/contact/.

10.3.3 **No Template Control** showing amplification

- Amplification of COVID-19 in a No Template Control (NTC) indicates contamination in one or more of the reagents, incorrect placement of a plate or tubes into the real-time PCR instrument, or pipetting errors.

None of the results can be trusted and re-testing by re-amplification should be performed. If the NTC fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the NTC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or by visiting www.codiagnostics.com/contact/.

## 11 LIMITATIONS

- This product is intended for research use only. Not intended for use in clinical diagnostics for its performance for diagnostic applications has not be established.
- Strict compliance with this document is required for optimal results. Please, always use the most recent version of this document. This can be downloaded for free at: www.codiagnostics.com/resources/instructions-for-use/ or by visiting www.codiagnostics.com/contact/.
- Use of this product is to be limited to trained and instructed personnel in real-time PCR techniques.
- Good laboratory practices are essential for the proper performance of this assay. It is also recommended that upon receipt of reagents a test run be performed to check the integrity, and performance of the reagents prior to testing on samples.
- Appropriate collection, transport, storage, and processing procedures of samples are required for optimal results.
- Do not use the **Logix Smart™ COVID-19** kit components directly on the specimens collected. Perform an appropriate nucleic acid extraction prior to using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the COVID-19 genome covered by this test kit may result in failure to detect the presence of the pathogens.

## 12 QUALITY CONTROL

In accordance with the Co-Diagnostics Inc.'s ISO 13485-certified Quality Management System, each lot of **Logix Smart™ COVID-19** kit is tested against predetermined specifications to ensure consistent product quality.

## 13 TECHNICAL ASSISTANCE

For technical assistance, please contact our Technical Support:

- Website: http://codiagnostics.com/contact/
- Email: info@codiagnostics.com
- Phone: 801-438-1036 ext. 04

## 14 REFERENCES

CDC. (2020, Feb 6). *2019 Novel Coronavirus > About 2019 Novel Coronavirus (2019-nCoV)*. Retrieved Feb 7, 2020, from Centers for Disease Control and Prevention: https://www.cdc.gov/coronavirus/2019-ncov/about/index.html

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Poritz, M. A., & Ririe, K. M. (2014). Getting Things Backwards to Prevent Primer Dimers. *The Journal of Molecular Diagnostics, 16*(2), 159-162. doi:10.1016/j.jmoldx.2014.01.001

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## 15 TRADEMARKS AND DISCLAIMERS

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

Product not available in all countries.

## 16 LEGEND OF PACKAGE SYMBOLS

| | |
|---|---|
| REF | Catalog number |
| LOT | Batch Code |
| CAP | Cap color |
| COMP | Component |
| CONT | Content/Volume |
| NUM | Number |
|  | Use-by-date |
|  | Contains sufficient for X tests/ reactions<br>X = 20 sample size<br>X = 100 regular size |
|  | Protect from light |
|  | Temperature limit |
|  | Consult Instructions for Use |
|  | Non-Sterile product – Do not sterilize |
|  | Manufacturer |
| RUO | Research Use Only |