IntelliPlex™ SARS-CoV-2 Detection Kit

Instructions for Use

REF 82303-U Σ 96 Reactions

IVD *In Vitro* Diagnostic Use

For use under an Emergency Use Authorization (EUA) only.

For Rx Use only

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IMPORTANT: Read the instructions carefully prior to Use

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1. INTENDED USE

The *IntelliPlex™ SARS-CoV-2 Detection Kit* is a molecular test based on reverse transcription-polymerase chain reaction (RT-PCR) in combination with πCode technology and the IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer with DeXipher software, and is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The *IntelliPlex™ SARS-CoV-2 Detection Kit* is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of PCR, PlexBio's instrument platform, and in vitro diagnostic procedures. The IntelliPlex SARS-CoV-2 Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. SUMMARY AND EXPLANATION

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-CoV-2), which has resulted in thousands of confirmed human infections in many countries including the United States. Cases of

asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

The *IntelliPlex™ SARS-CoV-2 Detection Kit* is a molecular *in vitro* diagnostic test that aids in the detection of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers, labeled oligonucleotide probes, and control material used for the *in vitro* qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

3. PRINCIPLES AND PROCEDURE

Coronaviruses are a large family of viruses that may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Severe Acute Respiratory Syndrome (SARS) and Coronavirus Disease 2019 (COVID-19). The COVID-19 is the infectious disease caused by the most recently discovered coronavirus, SARS-CoV-2. This new virus and disease were unknown before the outbreak began in December 2019. In a few months' time, COVID-19 has become a global pandemic, resulting in over four million cases world-wide. SARS-CoV-2 is the single-stranded RNA virus. Detection of SARS-CoV-2 nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage specimens are feasible due to the optimal primer and probe design in combination with πCode MicroDisc technology.

The assay includes primers/probe sets designed to detect SARS-CoV-2 specific target sequences including regions within the RdRP, E, and N genes.

πCode MicroDisc

πCode MicroDiscs are manufactured to generate more than 85,000 distinct circular image patterns for multiplexing applications. Each πCode has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. πCode tagged with different capture agents are pooled, enabling specific detection of multiple analytes in one-well reaction.

Detection Principle

The procedure is based on the following processes:

I. Viral RNA purified from acceptable respiratory specimens using the QIAamp Viral RNA Mini Kit.

II. RT-PCR amplification of viral RNA.

III. Hybridization of PCR amplicons with virus-specific probes conjugated to πCode MicroDiscs in a single well reaction.

IV. Incubation with SA-PE (Streptavidin-phycoerythrin) for fluorescent labeling.

V. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer.

4. MATERIALS PROVIDED

The *IntelliPlex™ SARS-CoV-2 Detection Kit* contains sufficient reagents for 96 tests. The kit components supplied are listed as follows.

1) SARS-CoV-2 KIT Primer Mix
Ref. No.: 20559-U
Quantity & Volume: 1 vial, 384 µL
Description: For RT-PCR amplification
Contents: ~4 µM Primer (including biotin-labeled primers)

2) SARS-CoV-2 KIT RT-PCR Buffer
Ref. No.: 20561-U
Quantity & Volume: 2 vials, 1 mL/vial
Storage: Store at -15°C to -25°C upon arrival
Description: For RT-PCR amplification
Contents: buffered solution containing $MgSO_4$ and dNTP

3) SARS-CoV-2 KIT RT-PCR Enzyme Mix
Ref. No.: 20560-U
Quantity & Volume: 1 vial, 96 µL
Storage: Store at -15°C to -25°C upon arrival
Description: For RT-PCR amplification
Contents: RT/Hot-Start Taq Mix (0.1 to 0.5 Units/µL): Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase and HotStart Taq DNA Polymerase, RNase Inhibitor

4) SARS-CoV-2 KIT πCode MicroDisc
Ref. No.: 20562-U
Quantity & Volume: 2 vials, 1 mL/vial
Description: For RT-PCR amplicon capture
Contents: πCode MicroDiscs (8-plex including RdRP, E, N, MS2, GUSB, Blank, SA-PE, Lot ID), Glycerol, Phosphate buffered saline, 0.1% Albumin-from bovine (Biological), <0.1% EDTA and <0.1% Sodium azide

5) SARS-CoV-2 KIT POS (Positive) Control
Ref. No.: 20563-U
Quantity & Volume: 3 vials, lyophilized
Description: Assay positive control; reconstituted

20 µL ddH_2O per vial prior to use. Single use only.
Contents: RNA representing SARS-CoV-2 E, N and RdRP gene mixed with human total RNA; preserved in RNA stable.

6) **SA-PE Solution**
Ref. No.: 20320-U
Quantity & Volume: 1 bottle, 10 mL/bottle
Description: Streptavidin-phycoerythrin for fluorescent signal acquisition
Contents: Phosphate buffered saline, 0.5% Streptavidin-phycoerythrin, 1% Albumin- from bovine (Biological), <0.1% Sodium azide

7) **Hy Buffer**
Ref. No.: 20565-U
Quantity & Volume: 1 bottle, 9.6 mL/bottle
Description: For assay hybridization
Contents: Saline-Sodium Phosphate-EDTA, <0.1% Sodium Azide as preservative

8) **10X Wash Buffer**
Ref. No.: 20546-U
Quantity & Volume: 2 bottles, 50 mL/bottle
Description: For πCode washing
Contents: Phosphate buffered saline containing surfactant and preservative

9) **NEG (Negative) Control**
Ref. No.: 20597-U
Quantity & Volume: 1 vial, 1.5mL
Description: Assay negative control
Contents: Nuclease-free water

10) **SARS-CoV-2 KIT Extraction Control**
Ref. No.: 20564-U
Quantity & Volume: 1 vial, 1 mL
Description: Assay external control
Contents: MS2 bacteriophage with RNA sequence serving as extraction control

11) **ddH_2O**
Ref. No.: 20548-U
Quantity & Volume: 1 vial, 1.5 mL/vial
Description: For reconstitution of SARS-CoV-2 KIT POS Control
Contents: Nuclease-free water

NOTE: POS Control, NEG Control and Hy Buffer refer to positive control, negative control and hybridization buffer, respectively.
The kit contains sufficient reagents for 3 independent test runs (including POS and NEG controls) and for a maximum of 96 tests.

5. MATERIALS AND EQUIPMENT

REQUIRED BUT NOT PROVIDED

Required products for compatibility with IntelliPlex kits:

- 96-well plate (PlexBio; Cat. No. 80025 or Greiner Bio-one; Cat. No. 655101)
- IntelliPlex™ 1000 πCode Processor (PlexBio; Cat. No. 80033)
- PlexBio 100 Fluorescent Analyzer (PlexBio; Cat. No. 80000)
- IntelliPlex™ Calibration Kit (PlexBio; Cat. No. 80035)
- U Tray (PlexBio; Cat. No. 80023)
- V Tray (PlexBio; Cat. No. 80024)
- DeXipher™ MD (PlexBio; Cat. No. 80051)
  - Lot ID, Completeness πCode MicroDiscs

Required components:

- QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52904/ 52906) or IntelliPrep Nucleic Acid Extraction Kit (PlexBio; Cat. No. 83003) + IntelliPrep Automated Nucleic Acid Extraction System (PlexBio; Cat. No. 80040)
- Clean tubes for PCR reaction (Gunster; Cat. No. MB-P08A or equivalent)
- Disposable gloves, powder-less
- Dedicated micropipette*
- Filter tips for micropipette*
- ddH_2O for dilution of 10X Wash Buffer
- Vortex mixer
- Micro-centrifuge
- Eppendorf® PCR Cooler or comparable (Recommended)
- Thermocycler: MiniAmp Thermal cycler (Thermo Fisher; Cat. No. A37834)
- Industrial Computer (Recommended: PlexBio; Cat. No. 80002)

* Use dedicated pipettes for sample purification, sample preparation, and sample hybridization. Do not share equipment between procedures. Pipettes should be accurate within 3% of the stated volume. Aerosol barrier or positive displacement DNA- and RNase-free tips must be used

6. WARNINGS AND PRECAUTIONS

- Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For emergency use only.
- For *in vitro* diagnostic use only (IVD).
- For Prescription Use Only (Rx).
- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories

certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab- biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Please read the package insert carefully prior to operation. The *IntelliPlex™ SARS-CoV-2 Detection Kit* is only for emergency use with a prescription, as an *in vitro* diagnostic test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
- False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.
- Separate, dedicated rooms and equipment for pre- and post-PCR process with a unidirectional workflow to avoid any contaminations is required.
- All pre-PCR steps should be carried out in the laminar flow hood to further reduce contamination risk.
- Do not use a kit or reagent past its expiration date.
- Sample preparation, RT-PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as those published by Clinical And Laboratory Standards Institute; clean all equipment and surface areas regularly (*e.g.*, The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution).
- Component reagents have been diluted optimally. Further dilution of the component reagents is not recommended.
- All chemicals, biological materials and human origin samples should be considered as potentially hazardous and/or infectious and should be treated accordingly.
- Some reagent contains EDTA and/or Sodium Azide in highly diluted concentration. Follow Good Laboratory Practices and Universal Precautions guidelines to avoid any risk.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens, and waste according to applicable central/federal, state, and local regulations.
- Wear powderless gloves and do not touch and make any markings on the bottom of the plate at any time, as fingerprints and markings would interfere with decoding and signal acquisition. Do not mark the top of the plate.
- General laboratory precautions should be taken:
  - Do not pipette by mouth.
  - Wear protective clothing (*e.g.*, disposable powderless gloves and laboratory coats) and eye protection.
  - Do not eat, drink or smoke in the laboratory.
  - Wash hands thoroughly after handling samples and reagents.
- Avoid RNase contamination:
  - Create an RNase-free working environment.
  - Wear gloves during all steps of the procedure.
  - Change gloves frequently.
  - Use only certified RNase-free sterile, disposable polypropylene tubes and filter strips.
  - Keep tubes closed whenever possible during the preparation.

- Use RNase removing product to clean bench surfaces, pipettes and other components used in the experiment.

- Material Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

7. REAGENT STORAGE, HANDLING AND STABILITY

Storage

The RT-PCR Buffer and RT-PCR Enzyme Mix of the *IntelliPlex™ SARS-CoV-2 Detection Kit* should be stored at -15°C to -25°C separately upon arrival.
Other kit components of the *IntelliPlex™ SARS-CoV-2 Detection Kit* should be stored at 2°C to 8°C.

Stability

Do not use the *IntelliPlex™ SARS-CoV-2 Detection Kit* when it is expired. All components are guaranteed up to the expiration date on the label if handled and stored under the recommended conditions.

Transportation

The shipping temperature for the *IntelliPlex™ SARS-CoV-2 Detection Kit* is at 2-8°C. If the kit package or components are incomplete, please contact PlexBio customer service (service@plexbio.com).

8. QUALITY CONTROL

The *Intelliplex™ SARS-CoV-2 Detection Kit* contains a series of internal control πCode MicroDiscs that monitor the specimen preparation, RT-PCR amplification, SA-PE incubation procedure, and background noise. These controls must always meet specifications and should have approximately the same intensity in each test well in the same test run. Otherwise, the test is invalid. The external controls (positive control and negative control) monitor the whole testing procedure to prevent false-positive or false-negative results. The test is considered invalid if any of the controls fail to meet the specified value.

Controls		Monitored Condition
Assay Performance Controls	Positive Control	RT-PCR amplification and all downstream procedures
	Negative Control	(Extraction contamination control), Non-specific amplification, cross-contamination
Sample Specific Controls	Reference Gene Control (GUSB)*	Specimen quality, RNA extraction, and all downstream procedures
	Extraction Control (MS2)	RNA extraction, RT-PCR amplification, and all downstream procedures
πCode MicroDisc Controls (monitored in each well-internal references)	Blank πCode MicroDiscs	Hybridization and washing conditions; fluorescence background
	SA-PE πCode MicroDiscs	Fluorescence labeling with SA-PE
	Lot ID πCode MicroDiscs	Lot expiration date
	Completeness of πCode MicroDiscs	Eight πCode MicroDisc types (with five or more MicroDiscs each) must be detected

*GUSB - Human glucuronidase Beta [GUSB] gene mRNA

9. INSTRUMENT AND SOFTWARE

Instrument

Refer to the instrument user manual for complete operation instructions (Thermo Fisher MiniAmp Thermal cycler, IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer).

Software Installation

The *IntelliPlex™ SARS-CoV-2 Detection Kit* has a designated Kit App and ENC file. The Kit App contains the πCode target assignments and the ENC file includes the lot number and expiration date. Please make sure you have the Kit App installed and the ENC file imported into DeXipher before your first assay run.

Kit App Installation

1. Log into www.plexbio.com and download the *IntelliPlex™ SARS-CoV-2 Detection Kit* App.
2. Click on the "Installer" in the APP folder and follow the instructions to complete the Kit App installation.

NOTE:
The Kit App only needs to be installed once. Version updates will be notified by customer service.

ENC File Installation

1. Log into www.plexbio.com and download the *IntelliPlex™ SARS-CoV-2 Detection Kit* ENC file. Each kit lot number will have a unique ENC file, so you will need to download a new ENC file each time you purchase a kit with a different lot number. Make sure to select the ENC file with the lot number that

corresponds to your kit.

2. Save the ENC file to your computer.
3. Follow the PlexBio 100 Fluorescent Analyzer User Manual to import the ENC file.

10. SPECIMENS

This kit is intended to be used with nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage specimens. Specimen collection, shipping and handling must follow the published guidelines from the Center of Disease Control and Prevention (CDC):
https://www.cdc.gov/coronavirus/2019- ncov/lab/guidelines-clinical-specimens.html

Specimen Collection

Follow specimen collection devices manufacturer instructions for proper collection methods.

Swab: Only synthetic fiber swabs with plastic shafts should be used. Do not use calcium alginate swabs or swabs with wooden shafts (inactivate some viruses and inhibit PCR testing). Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

Wash/Aspirate: Use 1 mL-1.5 mL of non-bacteriostatic saline (pH 7.0). Place specimen in a sterile viral transport media tube.

BAL: Samples should be collected into a sterile, leakproof collection cup or dry container without preservative matrix.

Specimen Transport

Suspected and confirmed SARS-CoV-2 patient specimens, cultures, or isolates must be packed and shipped according to UN 3373 Biological Substance, Category B, and in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations. Personnel must be trained to pack and ship according to the regulations and in a manner that corresponds to their function-specific responsibilities.

Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at -70°C or lower, ship overnight on dry ice.

Specimen Storage

If samples cannot be processed immediately upon receipt in the laboratory, specimens can be stored at 2-8°C for up to 72 hours after collection. If longer storage is expected due to a delay in processing or shipping, store specimens at -70°C or lower.

Purification and Storage of Extracted RNA

Before extraction, add 10 µL SARS-CoV-2 KIT Extraction Control to each specimen.

QIAamp Viral RNA Mini Kit

Purify samples using QIAamp Viral RNA Mini Kit according to manufacturer's instruction. The minimum specimen volume needed for purification processing is 140 µL, eluted in 50 µL buffer.

IntelliPrep Nucleic Acid Extraction Kit Purify samples using IntelliPrep Nucleic Acid Extraction Kit according to manufacturer's instruction. Sample extraction requires using the IntelliPrep Automated Nucleic Acid Extraction System. The specimen volume needed for purification processing is 400 µL, eluted in 50 µL buffer.

The NEG Control is included in the extraction process to monitor for contamination and is carried throughout the complete workflow including RT-PCR. A volume of 140 µL NEG Control is required for purification using QIAamp Viral RNA Mini Kit. A volume of 400 µL NEG Control is required for purification using IntelliPrep Nucleic Acid Extraction Kit. **Extraction Control (MS2) must not be added to the NEG Control**. Elute in 50 µL buffer.

Extracted RNA can be stored at 2°C to 8°C for up to 4 hours, or at -15°C to -25°C for up to 7 days. Long term storage is not recommended.

11. ASSAY PROCEDURE

Warning: Read the instructions carefully and follow every step of the assay protocol correctly.

Important Handling Instructions:
Separate, dedicated areas and equipment for sample purification, sample preparation and sample hybridization must be used. Equipment (including lab coats) must not be shared between areas. All equipment and surface areas should be cleaned before and after each run (*e.g.*, using a 0.5 – 1 % Sodium hypochlorite solution). All work should be performed according to approved guidelines such as those published by Clinical and Laboratory Standards Institute.

11.1 RT-PCR Amplification

1. If stored below -20°C, thaw purified samples on ice (4°C).

Procedure	Wash Buffer Consumption (mL)
Self-test	50
DNA/RNA program (1 lane, up to 8 tests)	150
DNA/RNA program (12 lanes, up to 96 tests)	535

2. Label RT-PCR tubes with unique numbers/names assigned. Include one tube for Positive Control and one tube for Negative Control.
   Positive Control must be reconstituted with 20 µL ddH_2O before use. Positive Control vials are single use only. Discard of unused leftovers.

3. Prepare the PCR reaction.

For each PCR reaction:

SARS-CoV-2 KIT RT-PCR Enzyme Mix	1 µL
SARS-CoV-2 KIT RT-PCR Buffer	20 µL
SARS-CoV-2 KIT Primer Mix	4 µL
Sample/PC/NC	15 µL
Total volume	40 µL

NOTE:

- The amount of RT-PCR reaction mix and primer mix required for a Master Mix depends on the number of reactions. Always prepare a surplus.
- Both POS Control and NEG Control are required for test validity and report generation and must be included in each assay run.

4. Mix by tapping the tubes and spin down before placing the tubes on the thermocycler. Set up the PCR program conditions as shown below:

Step	Temp.	Time	Cycles
RT	55˚C	15 min	1
Initial Denaturation	95˚C	2 min	1
Denaturation	95˚C	15 sec	36
Annealing/ extension	60˚C	30 sec	
	4˚C	∞	1

NOTE: *Ramp rate: 3 °C/sec (ABI MiniAmp)*

11.2 DNA Hybridization and SA-PE Reaction

1) **Prepare 1X Wash Buffer:** Transfer 50mL of the 10X Wash Buffer to the IntelliPlex 1000 πCode Processor 1L Wash Buffer bottle and add 450 ml ddH_2O. Mix by swirling.

   NOTE: The prepared 1X Wash Buffer can be used for up to one week.

   IntelliPlex 1000 πCode Processor Wash Buffer consumption:

   Required SA-PE Solution by Lane(s):

2) **Add 20 µL πCode MicroDisc to 96-well plate**: Mix by vortexing the **SARS-CoV-2 KIT πCode** for 10 seconds, then add 20 µL of the πCode to each well directly. Vortex the tube of πCode every four wells in between dispensing to ensure homogeneous suspension.

   NOTE: Each amplified PCR product (including samples, POS and NEG control) should be added into wells respectively in order of A1, B1…H1 and followed by A2, B2…H2 and so on.

3) **Dispense 100 µL of Hy Buffer** to each well.

4) Spin down the RT-PCR products.

5) **Denature the RT-PCR products** on the thermocycler by heating at 95˚C for 7 minutes followed by immediate cooling at 4˚C (Ramp rate: 100%) immediately.

   NOTE: Pay attention to the lid temperature of thermocycler while taking out the denatured PCR products.

6) Spin down the RT-PCR products and keep PCR products on ice (4˚C; *e.g.*, in Thermocycler or use Eppendorf® PCR Cooler or comparable). Use immediately (within 1 hour after denaturation).

7) **Add 20 µL of each freshly denatured sample** to corresponding well of 96-well plate (containing Hybridization buffer and πCode MicroDisc).

8) **Add 20 µL freshly denatured Positive Control** sample to corresponding well.

9) **Add 20 µL freshly denatured Negative Control** sample to corresponding well.

10) **Pipet the required volume of SA-PE solution** into the SA-PE solution tank (V Tray).

Number of Processed Lane(s)	Required SA-PE Solution (µL)
1	900
2	1300
3	1700
4	2100
5	2500
6	2900
7	3600
8	4000
9	4400
10	4800
11	5200
12	5600

11) **Run hybridization and wash:** This assay uses the **DNA/RNA program** in the **Molecular Assay** window of the IntelliPlex 1000 πCode Processor. Refer to the IntelliPlex 1000 πCode Processor operation manual and follow the instructions to run the built-in assay program (Homepage/ Molecular Assay/ Well Selection/ DNA/RNA/ Confirm procedure conditions / Start Running). The plate will be ready for decoding once the program finished (~60 minutes).

NOTE:

- SA-PE solution should be kept in the dark.
- **Do not** reuse the leftover SA-PE solution and V Tray. Replace a new V Tray with every assay run.
- **Do not** open the door when the instrument is in operation.
- The kit contains sufficient reagents for 3 independent test runs (including POS and NEG controls) and for a maximum of 96 tests. Please note that the included Wash Buffer is only sufficient for up to three independent runs. Additional Wash buffer can be ordered from PlexBio (Ref. No: 80210).

11.3 Image Decoding and Fluorescent Detection

1. Follow the PlexBio 100 Fluorescent Analyzer User Manual to set up the analysis.

   NOTE:

   - PlexBio 100 Fluorescent Analyzer must be calibrated regularly (once per month) using the IntelliPlex™ Calibration Kit .
   - Check that the correct ENC file has been imported.

2. Launch DeXipher to run the qualitative assay.
3. Mark the wells for sample, positive and negative controls.
4. Enter sample information and assay name. Place the plate into the device with the correct orientation as shown on the screen.
5. The raw data will be analyzed through the kit ENC to generate the genotype call report.

   NOTE:

   - A single run can include from 2 to 96 tests (including POS and NEG controls) per 96 microwell plate.

Step (for 94 specimens)	Time requirements	Description
Sample Extraction	60 minutes	QIAamp Viral RNA Mini Kit
Setup/ Run RT-PCR	90 minutes	MiniAmp Thermal cycler
Hybridization/ SAPE	60 minutes	IntelliPlex™ 1000 πCode Processor
Analyzing Results	60 minutes	PlexBio 100 Fluorescent Analyzer + DeXipher software

12. INTERPRETATION OF RESULTS

The DeXipher software will analyze specimen samples only if the external controls (Positive Control and Negative Control) and internal controls (Reference Gene Control, Blank and SAPE Monitor Control) are all shown as “Pass”. Failed Positive or Negative Control renders the whole assay invalid. Failed Reference Gene Control, Blank or SAPE Monitor Control renders the affected sample invalid.

Please also refer to the chapter “Disclaimer and Limitations” and “Troubleshooting” for additional information.

The RdRP, E, and N targets are all specific to SARS-CoV-2. Detection of any one, two, or three targets is considered a valid positive result. Only qualitative results of SARS-CoV-2 Detected/Not Detected is shown on the test report.

Result Interpretation

Reported Result	Interpretation	Action
"Detected"	SARS-CoV-2 positive	Report results to health care provider and appropriate public health authorities
"Not Detected"	SARS-CoV-2 negative	
No result reported	Assay was not valid due to external or internal control failure	Troubleshoot to address the control issue or contact PlexBio customer service team; retest or obtain new specimen as necessary.

13. DISCLAIMERS AND LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
- The performance of this test was evaluated using the procedures provided in this product insert. Alteration from the procedure may affect test performance.
- The performance of *IntelliPlex™ SARS-CoV-2 Detection Kit* was established using nasopharyngeal swab samples. Anterior nasal, oropharyngeal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage are also considered acceptable specimen types for use with the *IntelliPlex™ SARS-CoV-2 Detection Kit* but performance has not been established.
- A negative test result means the *IntelliPlex™ SARS-CoV-2 Detection Kit* was unable to detect the virus in the sample. It does not preclude the possibility that the specimen did in fact contain the virus. Only samples with detectable amounts of the virus matching the reference sequences are detected; false negative test results may be due to experimental errors or other causes. Interpretation of the results should consider these possibilities.
- A positive test result means that the *IntelliPlex™ SARS-CoV-2 Detection Kit* was able to determine SARS-CoV-2 in the sample. False positive test results may be caused by experimental errors or other causes. Interpretation of the results should consider these possibilities.
- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the RdRP, E, and N primer/probe sets of the *IntelliPlex™ SARS-CoV-2 Detection Kit*. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- Laboratories are required to report all positive results to the appropriate public health authorities.

14. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The *IntelliPlex™ SARS-CoV-2 Detection Kit* Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas. However, to assist clinical laboratories using the *IntelliPlex™ SARS-CoV-2 Detection Kit*, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using *IntelliPlex™ SARS-CoV-2 Detection Kit* will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using *IntelliPlex™ SARS-CoV-2 Detection Kit* will use your product as outlined in the authorized labeling. Deviations from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use *IntelliPlex™ SARS-CoV-2 Detection Kit* are not permitted.

C. Authorized laboratories that receive *IntelliPlex™ SARS-CoV-2 Detection Kit* will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using *IntelliPlex™ SARS-CoV-2 Detection Kit* will have a process in place for reporting test results to healthcare providers and relevant public health authorities.

E. Authorized laboratories will collect information on the performance of *IntelliPlex™ SARS-CoV-2 Detection Kit* and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUAReporting@fda/hhs.gov) and PlexBio Co. Ltd. (Adverse event reporting: https://www.plexbio.com/intelliplex%E2%84%A2-sars-cov-2-detection-kit) if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the

established performance characteristics of your product .

F. All laboratory personnel using *IntelliPlex™ SARS-CoV-2 Detection Kit* must be appropriately trained in molecular techniques and use appropriate laboratory and personal protective equipment when handling this kit and use *IntelliPlex™ SARS-CoV-2 Detection Kit* in accordance with the authorized labeling.

G. PlexBio Co. Ltd., authorized distributors, and authorized laboratories using *IntelliPlex™ SARS-CoV-2 Detection Kit* will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

15. ANALYTICAL PERFORMANCE

Limit of Detection (Analytical Sensitivity)

QIAamp Viral RNA Mini Kit

Limit of detection (LoD) was determined as the lowest concentration of SARS-CoV-2 that can be detected at a ≥95% positive rate with the *IntelliPlex™ SARS-CoV-2 Detection Kit*. Samples were prepared by spiking target RNA (i.e., produced by transfecting HEK-293 cells with plasmids expressing either the N, E or RdRP mRNA of SARS-CoV-2) at different concentration into confirmed negative nasopharyngeal (NP) swabs in viral transport media (VTM). A dilution series ranging from 420 copies/mL – 70 copies//mL with six replicates per concentration were tested to determine the preliminary LoD.

The final LoD concentration was confirmed by testing 20 contrived replicates using SARS-CoV-2 Reference Material (SeraCare; AccuPlex™ SARS-CoV-2 Reference Material Kit, Cat 0505-0126).

The LoD for the *IntelliPlex™ SARS-CoV-2 Detection Kit* is 140 copies/mL using QIAamp Viral RNA Mini Kit.

Replicate	Median Fluorescence Intensity (MFI)*			Detection of SARS-CoV-2
	RdRP	E	N	
140 copies/mL				
1	14013	9625	18421	Positive
2	9821	7770	6381	Positive
3	13945	3764	14112	Positive
4	11003	12579	13018	Positive
5	16604	5417	10074	Positive
6	15413	5978	12942	Positive
7	15436	9415	8534	Positive
8	15072	11946	19621	Positive
9	18751	6154	13692	Positive
10	12918	3859	11761	Positive
11	12087	12189	21597	Positive
12	8045	9852	15830	Positive
13	9613	7046	9708	Positive
14	10479	7491	7905	Positive
15	6427	8679	7054	Positive
16	6196	0	4463	Positive
17	15539	12060	15610	Positive
18	13324	13542	16762	Positive
19	7935	13807	16372	Positive
20	5048	12696	16204	Positive
Average MFI	**11883**	**8693**	**13003**	**20/20 (100%)**

*MFI – values after subtraction of the threshold

Replicate	Median Fluorescence Intensity*			Detection of SARS-CoV-2
	RdRP	E	N	
70 copies/mL				
1	493	0	0	Positive
2	0	0	0	Negative
3	0	2669	4301	Positive
4	0	0	11	Positive
5	0	0	0	Negative
6	0	0	0	Negative
7	3747	423	0	Positive
8	1535	307	0	Positive
9	0	0	3134	Positive
10	0	506	862	Positive
11	524	693	10146	Positive
12	1609	1440	0	Positive
13	947	6497	8184	Positive
14	0	0	0	Negative
15	1130	827	1874	Positive
16	0	1600	8950	Positive
17	0	658	0	Positive
18	4898	0	0	Positive
19	850	0	0	Positive
20	0	1012	2758	Positive

Replicate	Median Fluorescence Intensity*			Detection of SARS-CoV-2
	RdRP	E	N	
70 copies/mL				
Average MFI	**787**	**832**	**2011**	**16/20 (80%)**

*MFI – values after subtraction of the threshold

IntelliPrep Nucleic Acid Extraction Kit

Limit of detection (LoD) for IntelliPrep Nucleic Acid Extraction Kit assay was determined as the lowest concentration of SARS-CoV-2 that can be detected at a ≥95% positive rate with the IntelliPlex SARS-CoV-2 Detection Kit. Samples were prepared by spiking viral SARS-CoV-2 RNA, derived from a natural sample, at different concentrations into confirmed negative nasopharyngeal (NP) swabs in transport media.

A dilution series ranging from 400 copies/mL to 50 copies/mL with five replicates per concentration was tested to determine the preliminary LoD.

	400 copies/ml	200 copies/ml	100 copies/ml	50 copies/ml	0 copies/ml
Detected / Total	**5/5**	**5/5**	**5/5**	**4/5**	**0/0**
Sample Positive for SARS-COV-2	**100%**	**100%**	**100%**	**80%**	**0%**

The final LoD was then confirmed testing 20 contrived specimen, prepared as described above, at a concentration of 1 x LoD. The LoD for the *IntelliPlexTM SARS-CoV-2 Detection Kit* is 100 copies/mL using IntelliPrep Nucleic Acid Extraction Kit.

Replicate	Median Fluorescence Intensity (MFI)*			Detection of SARS-CoV-2
	RdRP	E	N	
100 copies/mL				
1	3717	1327	1192	Positive
2	1660	2888	3344	Positive
3	2633	1193	1632	Positive
4	3191	1984	3675	Positive
5	3094	0	2104	Positive
6	2006	3342	2964	Positive
7	3766	2766	712	Positive
8	2702	1853	3006	Positive
9	3783	2983	2745	Positive
10	5884	3085	3149	Positive
11	5195	2633	3583	Positive
12	3965	2651	3231	Positive
13	2682	3215	4115	Positive
14	4712	3926	1837	Positive
15	3043	2651	3462	Positive
16	4845	2334	2007	Positive
17	3521	2946	2316	Positive
18	7513	4619	1586	Positive
19	3397	2004	3487	Positive
20	5544	2448	1186	Positive
Average MFI	**3842**	**2542**	**2566**	**20/20 (100%)**

*MFI – values after subtraction of the threshold

Inclusivity (Analytical Reactivity)

BLASTn analysis query alignments were performed with the SARS-CoV-2 E, N and RdRP oligonucleotide primer and probe sequences with full length or near-full-length (>29 kb) nucleic acid sequences for SARS-COV-2 in NCBI's Severe Acute Respiratory Syndrome Coronavirus 2 Data Hub (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/) The analysis demonstrated the following:

- 1 mismatch in the "N F Primer" binding site (GenBank: MT246456, MT263410, MT291836 and MT293159)
- 2 mismatches in the "N R Primer" binding site (GenBank: MT263411),
- 3 mismatches in the "N R Primer" (GenBank: MT258379, MT259250, MT259263, MT246470, MT246451, MT233522)
- 1 mismatch in the "E probe" (GenBank: MT263433).

The primers and probe set targeting the RdRP had no mismatch against any of the published sequences.

Despite these mutations in SARS-CoV-2, the IntelliPlex assay is still expected to detect all SARS-CoV-2 strains. Because the assay detects 3 targets that are specific to SARS-CoV-2, even if new or not previously reported nucleotide mutations affect amplification/detection of one of the targets, the presence of the other two targets can still generate a valid positive result.

Cross Reactivity (Analytical Specificity)

Cross-reactivity of the *IntelliPlex™ SARS-CoV-2 Detection Kit* was evaluated by *in silico* analysis and by performing wet lab testing.

BLASTn analysis queries of the *IntelliPlex™ SARS-CoV-2 Detection Kit* primers and probes were performed against the sequences of the organisms listed in the table below.

The *in silico* analysis predicted that SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the RdRP, E, and N primer/probe sets of the IntelliPlex SARS-CoV-2 Detection Kit.

The RdRP F primer showed homology > 80% to human coronavirus HKU1 (90.9%) and OC43 (90.9%). The RdRP R primer showed homology > 80% to human coronavirus HKU1 (82.7%), OC43 (82.7%), NL63 (89.6%) and MERS coronavirus (82.7%). The RdRP probe showed homology >80% to HKU1 (81.2%), OC43 (81.2%) and MERS coronavirus (87.5%).

The reverse primer for the Reference Gene Control (GUSB R Primer) showed homology > 80% to *Pseudomonas aeruginosa* sequences, and the forward primer for SARS-CoV-2 E gene showed homology > 80% to *Pseudomonas aeruginosa* sequences. None of the other amplification primers showed homology > 80% to any of the sequences included in the analysis.

The RdRP probe sequence showed high homology (>80%) to off-target sequences. Specifically, the probe is 100% complementary to *Candida albicans* and shows homology >80% to *Mycoplasma pneumoniae*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. In addition, the GUSB probe sequence showed high homology (>80%) to off-target sequences. The probe is >80% complementary to *Candida albicans* and *Pseudomonas aeruginosa*, and *Pneumocystis jirovecii*.

High priority pathogens from the same genetic family		
Organism	**Taxid**	**Notes**
SARS coronavirus	694009	100% homology to RdRP F Primer; 96.5% homology to RdRP R Primer; 93.8% homology to RdRP Probe; 100% homology to E F Primer; 100% homology to E R Primer; 83.3% homology to E Probe; 90.9% homology to N F Primer; 90.9% homology to N R Primer; 80% homology to N Probe;
Human coronavirus 229E	11137	No homology >80%
Human coronavirus OC43	31631	90.9% homology to RdRP F Primer; 82.7% homology to RdRP R Primer; 80.2% homology to RdRP Probe
Human coronavirus HKU1	290028	90.9% homology to RdRP F Primer; 82.7% homology to RdRP R Primer; 80.2% homology to RdRP Probe
Human coronavirus NL63	277944	89.6% homology to RdRP R Primer
MERS coronavirus	1335626	82.7% homology to RdRP R Prime; 87.5% homology to RdRP Probe

High priority organisms likely in the circulating area		
Organism	**Taxid**	**Notes**
Adenovirus 4	28280	No homology >80%
Adenovirus 7	10519	No homology >80%
HMPV	162145	No homology >80%
Human parainfluenza 1 virus	12730	No homology >80%
Human parainfluenza 2 virus	1979160	No homology >80%
Human parainfluenza 3 virus	11216	No homology >80%
Human parainfluenza 4a virus	11224	No homology >80%
Human parainfluenza 4b virus	11226	No homology >80%
Human Influenza A Virus	11320	No homology >80%
Influenza B virus	11520	No homology >80%
Human enterovirus EV68	42789	No homology >80%
Human respiratory syncytial virus	11250	No homology >80%
Rhinovirus	12059	No homology >80%
Chlamydia pneumoniae	83558	No homology >80%
Haemophilus influenzae	727	No homology >80%
Legionella pneumophila	446	No homology >80%
Mycobacterium tuberculosis complex	77643	81.3% homology to MS2 probe

High priority organisms likely in the circulating area		
Organism	**Taxid**	**Notes**
Streptococcus pneumoniae	1313	No homology >80%
Streptococcus pyogenes	1314	No homology >80%
Bordetella pertussis	520	No homology >80%
Mycoplasma pneumoniae	2104	80% homology to RdRP probe
Pneumocystis jirovecii	42068	81.3% homology to GUSB probe
Candida albicans	5476	100% homology to RdRP probe; 93.8% homology to GUSB probe
Pseudomonas aeruginosa group	136841	80% homology to RdRP probe; 81% homology to E F primer; 85% homology to GUSB R primer; 81.3% homology to GUSB probe
Staphylococcus epidermidis	1282	93.8% homology to RdRP probe

Cross-reactivity wet testing was performed to demonstrate that the *IntelliPlex™ SARS-CoV-2 Detection Kit* does not react with other organisms that are reasonably likely to be encountered in the clinical specimen. The study included the organisms listed below using the Zeptometrix NATtrol Respiratory Panel 2 (Catalog Number: NATRVP2-BIO), NATtrol BC/GM Panel (Catalog Number: NATBCGN-NNS), Zeptometrix: NATtrol BC/GP Paneland (Catalog Number: NATBC/GP-NNS) and *C. albicans* strain from the Taiwan Bioresource Collection and Research Center (BCRC).

All organisms were tested in triplicate and at high pathogen concentrations (>10^6 copies/ assay).
None of the wet-tested organisms produced a positive signal for the *IntelliPlex™ SARS-CoV-2 Detection Kit*.

Organism	Source	Results
Human coronavirus 229E	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human coronavirus OC43	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human coronavirus HKU1	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human coronavirus NL63	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human Metapneumovirus (hMPV)	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 1	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 2	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 3	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 4	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza A -H1	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza A -H1 2009	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza A -H3	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza B	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Respiratory syncytial virus	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Rhinovirus	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Chlamydia pneumoniae	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Streptococcus pyogenes	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Bordetella pertussis	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Pseudomonas aeruginosa	Zeptometrix (NATBCGN-NNS)	No Cross Reactivity
Staphylococcus epidermidis	Zeptometrix (NATBC/GP-NNS)	No Cross Reactivity
Mycoplasma pneumoniae	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Candida albicans	BCRC	No Cross Reactivity

16. CLINICAL STUDY

- **Testing on Contrived Sample**

The performance of the *IntelliPlex™ SARS-CoV-2 Detection Kit* was evaluated using contrived clinical nasopharyngeal (NP) swab specimens. Samples were prepared by spiking SARS-CoV-2 Reference Material (SeraCare; AccuPlex™ SARS-CoV-2 Reference Material Kit, Cat. No. 0505-0126) at different concentrations into individual, unique confirmed negative nasopharyngeal (NP) swab matrix. Negative NP swab samples were also tested. Samples were blinded and randomized for testing.

The results are shown below.

Performance Evaluation

Conc. RNA RdRP, E and N Gene	2x LoD (280 copies/mL)	1x LoD (140 copies/mL)	Negative
Number of NP Swabs	10	20	30
Detection Rate	10/10	20/20	0/30
Agreement with Expected Result (%)	100	100	100

- **Testing on Clinical Sample**

A clinical evaluation study of the *IntelliPlex™ SARS-CoV-2 Detection Kit* was conducted with de-identified natural leftover clinical nasopharyngeal swab specimens that were previously collected for SARS-CoV-2 testing (SARS-COV-2 Validation Panel; Boca Biolistics). All specimens were evaluated with an FDA EUA authorized comparator assay (Panther Fusion SARS-CoV-2 Assay; Hologic, Inc.). A total of 62 patient specimens (30 positive and 32 negative for SARS-CoV-2) were included in the study.

All specimen testing with the *IntelliPlex™ SARS-CoV-2 Detection Kit* was conducted blinded and followed the instruction provided in the IFU. The *IntelliPlex™ SARS-CoV-2 Detection Kit* detected SARS-CoV-2 in 30 of 30 positive specimens and reported 31 of 32 negative specimens.

	IntelliPlex SARS-CoV-2 Detection Kit			
	positive	negative	Total	Agreement
Comparator positive	30	0	30	100% (30/30)
Comparator negative	1	31	32	96.875 % (31/32)

THE POSITIVE PERCENT AGREEMENT (PPA) FOR INTELLIPLEX SARS-COV-2 DETECTION KIT IS 100 % (30/30) AND THE NEGATIVE PERCENT AGREEMENT (NPA) FOR INTELLIPLEX SARS-COV-2 DETECTION KIT IS 96.875 % (31/32).

17. TROUBLESHOOTING

- The troubleshooting steps listed below address possible problem causes and solutions that could be experienced during the assay procedures. If the problem persists after completing the recommended steps in the table provided below, please contact PlexBio customer service immediately for further assistance.
- Specimens may be retested using the previously extracted nucleic acid if stored according to requirements. In the event re-extraction of viral RNA is required, access the original, properly stored sample and repeat the full assay procedure. If the repeat testing still results in a failure, it is recommended to obtain a new patient specimen.
- In the event of a Positive Control failure, the sample may be re-tested using the previously extracted nucleic acid and stored according to requirements. In the event of a Negative Control failure due to contamination, it is recommended to re-extract the original properly stored sample and repeat the full assay procedure. If the Negative Control failed because of πCode MicroDiscs failure, retesting using residual extracted specimen RNA is accepted. If the Reference Gene Control or Extraction Control has failed, extract new nucleic acid before testing. If testing is affected by specimen quality, a new specimen is required. If a πCode MicroDiscs control failed, retesting using residual extracted specimen RNA is accepted.

Problem	Possible Cause	Recommendations
No Valid Assay Assigned	1. No plate inserted. 2. Plate inserted in wrong orientation. 3. No assay APP installed. 4. No ENC file imported. 5. Two or more lots of reagent used.	1. Confirm plate is inserted and repeat reading. 2. Confirm orientation of plate and repeat reading. 3. Install assay APP and repeat reading. 4. Import ENC file and repeat reading. 5. One reagent lot used at a time.

Problem	Possible Cause	Recommendations
Positive Control Fail	1. No Positive Control added.	1. Ensure Controls are added. Ensure proper reconstitution of Positive Control as described. Repeat testing using residual extracted sample for RT-PCR.
	2. RNase contamination.	2. Ensure all operating procedures are followed correctly and work environment is free of RNase. Repeat testing using newly extracted sample.
	3. Assay did not work.	3. Make sure all the assay procedures are followed correctly. Ensure all components are stored at required storage conditions. Repeat testing using residual extracted sample for RT-PCR. If there is a general problem with assay performance, obtain new assay kit.
	4. Wrong PC well selected.	4. Choose the correct PC well and repeat reading.
	5. "πCode MicroDiscs Combination", "Blank Control", "πCode MicroDiscs Count", or "SAPE Monitor Control" failed	5. See section below for more details. Repeat testing using residual extracted sample for RT-PCR.

Problem	Possible Cause	Recommendations
Negative Control Fail	1. Cross-contamination between samples	1. Clean all surfaces and equipment. Instruction in Package Insert on utilizing different rooms and unidirectional workflow must be followed. Repeat testing using newly extracted sample.
	2. Wrong NC well selected.	2. Choose the correct NC well and repeat reading.
	3. "πCode MicroDiscs Combination", "Blank Control", "Reference Gene Control", "πCode MicroDiscs Count", or "SAPE Monitor Control" failed	3. See section below for more details. Repeat testing using residual extracted sample for RT-PCR.
πCode MicroDiscs Combination Fail	1. πCode MicroDiscs from a different assay/ lot are used.	1. Use πCode MicroDiscs provided with the *IntelliPlex™ SARS-CoV-2 Detection Kit* and ensure the lot-ENC is available.
	2. Missing π Code MicroDiscs due to wrong operation	2. Refer to "πCode MicroDiscs Count Fail" below.
πCode MicroDiscs Count Fail	1. πCode MicroDiscs are not properly dispersed in the well.	1. Re-disperse the microplate using IntelliPlex 1000 Processor, and repeat reading.
	2. Not enough πCode MicroDiscs added to well.	2. Ensure πCode MicroDiscs are well-mixed with proper amount added. Repeat using residual extracted sample for RT-PCR.
	3. Microbes exist in Wash buffers.	3. Use freshly prepared wash buffer and ddH_2O for hybridization to reduce πCode MicroDiscs loss rate. Repeat using residual extracted sample for RT-PCR.
	4. Instruments error or malfunction.	4. Contact PlexBio Customer Service.

Problem	Possible Cause	Recommendations
SAPE Monitor Control Fail	1. No SA-PE was added or insufficient SA-PE solution for dispensing.	1. Make sure all the assay procedures are followed correctly. Calculate sufficient SA-PE solution volume for dispensing. Repeat testing using residual extracted sample for RT-PCR.
	2. SA-PE solution deactivated.	2. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date.
	3. Incorrect tested lanes of microplate selected for SA-PE solution dispensing.	3. Repeat assay using residual extracted sample for RT-PCR and make sure lanes are selected correctly.
Blank Control Fail	1. Wrong hybridization conditions.	1. Ensure correct hybridization program is selected. Repeat testing using residual extracted sample for RT-PCR.
	2. Residues of SA-PE solution in wells after hybridization.	2. Ensure all buffers (Wash buffer and ddH_2O) on IntelliPlex 1000 Processor are fresh-made and sufficient for washing procedures. Repeat testing using residual extracted sample for RT-PCR.
	3. PlexBio 100 Fluorescent Analyzer is not calibrated.	3. Perform calibration on PlexBio 100 Fluorescent Analyzer. Repeat testing using residual extracted sample for RT-PCR.
	4. Markings on plates.	4. Do not make any marking on the plate. Repeat testing using residual extracted sample for RT-PCR.

Problem	Possible Cause	Recommendations
Extraction Control Fail	1. The Extraction Control was not correctly added to the specimen	1. Follow the instruction provided in the Package Insert. Repeat testing using newly extracted sample.
	2. Problem during nucleic acid purification/ extraction.	2. Follow the instruction provided by the manufacturer of the nucleic acid purification kit. Ensure all buffers are freshly prepared. Repeat testing using newly extracted sample.
Reference Gene Control Fail	1. Poor specimen sample quality	1. Specimen was not collected, transported, or stored according to requirements.
	2. RNA purification failed or PCR inhibitors existed.	2. Follow instructions of sample extraction carefully. Ensure required temperature ranges and centrifugation needs are complied. Ensure complete removal of ethanol. Repeat testing using newly extracted sample.
	3. PCR procedures are not performed correctly.	3. Make sure all PCR procedures are followed correctly. Do not to use expired materials or mixed lots of reagents. Ensure storage conditions are correct. Repeat testing with residual extracted sample for RT-PCR.
	4. RNase contamination.	4. Ensure all the operating procedures are followed correctly. Ensure work environment is free of RNase. Repeat testing using newly extracted sample.
	5. Hybridization did not work.	5. Make sure all the assay procedures are followed correctly. Ensure samples are freshly heat-denatured. Repeat testing using residual extracted sample for RT-PCR.

18. SYMBOLS

Symbol	Explanation	Symbol	Explanation
IVD	In-vitro diagnostic use	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
	Contains sufficient for <n> tests		Date of Manufacture

19. CONTACT INFORMATION AND PRODUCT SUPPORT

For technical and product support, contact:

service@plexbio.com

Service hotline:

+886-2627-5878

Office hour: 09:00-18:00 (GMT+8)

U.S. Technical and Product Support:

+1 415-310-6025

Product support website:

www.plexbio.com

Notice to User

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