IntelliPlex™ SARS-CoV-2 Detection Kit

REF **82303** **96 Reactions**

CE IVD **For In-Vitro Diagnostic Use**

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IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPlex SARS-CoV-2 Detection Kit is a qualitative detection of SARS-CoV-2 virus in patients that may or may not exhibit symptoms of respiratory tract infection (i.e. fever, cough, shortness of breath). For the use with IntelliPlex 1000 πCode Processor and PlexBio™ 100 Fluorescent Analyzer, the kit detects the presence of N, RdRp, and E genes of SARS-CoV-2 in RNA extracted from nasopharynx of upper respiratory tract. Viral RNA needs to be purified before amplification by reverse transcription-polymerase chain reaction (RT-PCR). The detection of viruses is intended to assist clinicians in the management of infection treatments.

2. INTRODUCTION

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 pandemic, resulting in over a hundred thousand cases. SARS-CoV-2 is the single-stranded RNA virus. Detection of SARS-CoV-2 from the specimen of nasopharyngeal swab is feasible due to the optimal primer and probe design in combination with πCode MicroDisc technology.

3. TECHNOLOGICAL PRINCIPLES

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 processes listed as follows:

I. Viral RNA purified from nasopharyngeal swab.
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 for fluorescent labeling.
V. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer.

4. WARNINGS AND PRECAUTIONS

- For in-vitro diagnostic use.
- This assay kit should be used by qualified laboratory personnel only.
- 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.**
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- Material Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

5. PRODUCT USE LIMITATIONS

The recommendations and procedures must be followed to prevent false results and contamination.

- Virus with mutations/sequences different from the reference sequence may not be identified correctly.
- Specimen collection, storage, purification, and transport must comply to allow efficient amplification with the RT-PCR assay.

6. 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.

7. KIT COMPONENTS

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
   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
   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
   Quantity & Volume: 1 vial, 96 µL/vial
   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
   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 Control**
   Ref. No.: 20563
   Quantity & Volume: 3 vial, lyophilized
   Description: Assay positive control; reconstituted by 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
   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
   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
   Quantity & Volume: 2 bottles, 50 mL/bottle
   Description: For πCode washing
   Contents: Phosphate buffered saline containing surfactant and preservative

9. **NEG Control**
   Ref. No.: 20549
   Quantity & Volume: 1 vial, 120 µL
   Description: Assay negative control
   Contents: Nuclease-free water

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

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

8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

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)
- U Tray (PlexBio; Cat. No. 80023)
- V Tray (PlexBio; Cat. No. 80024)
- DeXipher™ MD (Required: PlexBio; Cat. No. 80051)

Required components:

- QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 50904), ConcertBio extraction kit or equivalent
- 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 (Recommended: MiniAmp Thermal cycler, Thermo Fisher)
- 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.

9. STORAGE, STABILITY AND TRANSPORTATION

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. Once opened, the reagent components are stable for 6 months or until the expiration date, whichever comes first.

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).

10. INSTRUMENT AND SOFTWARE

Instrument

Refer to the instrument user manual for complete operation instructions (Thermocycler, 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 **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 **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.

11. SPECIMENS

Specimen Collection

Follow specimen collection devices manufacturer instructions for proper collection methods. 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.

Specimen Transport

Suspected and confirmed SARS-CoV-2 patient specimens, cultures, or isolates must be pack 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

Specimens can be stored at 2-8°C for up to 72 hours after collection. If longer storage is expected, 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.

CAUTION: DO NOT vortex the **SARS-CoV-2 KIT Extraction Control**. Please tap the vial gently then centrifuge briefly prior to use.

Samples are purified by 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.

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.

12. 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.

12.1 RT-PCR Amplification

1. If stored below -20°C, thaw purified samples on ice (4°C).
2. Label RT-PCR tubes with unique numbers/names assigned. Include one tube for Positive Control and one tube for Negative Control.
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	4µL
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:

Temp. (˚C)	Time	Cycles
55	15 min	1
95	2 min	1
95	15 sec	36
60	30 sec	
4	∞	1

NOTE: *Ramp rate: 3 ˚C/sec (MiniAmp™; Cat. No. A37834).*

12.2 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:

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. **Add 20 µL πCode MicroDisc to 96-well plate**: Mix by vortexing the **SARS-CoV-2 KIT πCode** for 10 seconds (without pipetting), 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 products (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 up to 95˚C for 7 minutes then cooling down to 4˚C (Ramp rate: 100%) without delay.

   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).

    Required SA-PE Solution by Lane(s):

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

    - SA-PE solution should be kept in the dark.
    - **Do not** reuse the leftover SA-PE solution and V Tray tank. Replace a new V Tray with every assay run.
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.

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

12.3 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).
   - 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 well Microwell plate.

13. DISCLAIMERS

Negative Test Result

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.

Positive Test Result

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.

14. INTERPRETATION OF RESULTS

Make sure the results of external control (POS Control and NEG Control) and internal control (Reference Gene Control, SA-PE Monitor Control, πCode MicroDiscs Count, πCode MicroDiscs Combination, Extraction Control and Blank Control) are all shown as "Pass". Failed POS or NEG Control renders the whole assay invalid. Failed Internal Control renders the affected sample invalid. Please also refer to the chapter "Troubleshooting".

For additional information please refer to **"Product Use Limitations"** and **"Troubleshooting"** section of this manual.

Table 1. Result Interpretation

Test Result	Report Result	Interpretation
Virus Detected	Detected	Virus detected on the specified targeted region
Virus Not Detected	Not Detected	1. No Sample added 2. Sample titer below LoD
Invalid Assay	Blank	1. PCR Inhibition (presence of inhibitor in the sample) 2. SAPE Reaction Failed 3. Low sample RNA input or quality 4. Low πCode Disc Count (the πCode tube was not vortexed before pipetting) 5. No πCode Detected 6. Failed Blank πCode Control

15. ANALYTICAL PERFORMANCE

Limit of Blank (LoB)

The limit of blank (LoB) values were determined by performing 30 confirmed negative nasopharyngeal swab on 2 reagent lots and one set of instruments. The cutoff values of each targeted gene were determined by the measured maximum analytical signal intensity values, respectively.

Only "No Detected" results were observed in these SARS-CoV-2 negative samples.

Limit of Detection (LoD, Analytical Sensitivity)

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 at different concentration into confirmed negative nasopharyngeal (NP) swabs in viral transport media (VTM). Several dilution series of three replicates per concentration were carried out and tested to determine the preliminary LoD. The final LoD concentration was confirmed by testing 20 replicates.

The LoD (95% detection rate) for the IntelliPlex SARS-CoV-2 Detection Kit is 140 copies/mL.

Table 2. Summary of Limit of Detection Tested

RNA Conc. of SARS-CoV-2	280 copies/mL	140 copies/mL	70 copies/mL
Specimen Number	**20**	**20**	**20**
Positive for SARS-CoV-2	20 (100%)	19 (95%)	12 (60%)
Positive for N Gene	19 (95%)	15 (75%)	5 (25%)
Positive for E Gene	20 (100%)	19 (95%)	7 (35%)
Positive for RdRp Gene	20 (100%)	13 (65%)	4 (20%)

Note: One or more positive results of target (N, E and RdRp genes) show the positive result of SARS-CoV-2 detection.

Repeatability and Reproducibility

The repeatability and reproducibility of SARS-CoV-2 Detection Kit was evaluated across two reagent lots, two sites, two operators, two sets of instrument and five non-consecutive testing days. One operator performed two run per day for a total of 20 runs at one site. Repeatability and reproducibility was demonstrated with low level mutant (2x LoD) and high level mutant (6x LoD) spiked in HECK 293 cell line RNA. The correct call of the all testing level of each target gene was 100% (40/40) across all variance combined (i.e., site/instrument, operator, and day). Across all variance components (i.e., site/instrument, operator, and day), the overall coefficient of variation is smaller than 5% across all panel members.

Analytical Specificity

A search was carried out to assess the homology of oligos utilized in the **IntelliPlex SARS-CoV-2 Detection Kit** towards high priority pathogens from the same genetic family (except SARS) and high priority organism likely in the circulating area. All the pathogens are completed with BLAST analysis assay while some pathogens are validated by performing wet lab testing.

Table 3. BLAST Analytic Result of Cross Reactivity

Virus		
Human coronavirus HKU1 (taxid:290028)	Human coronavirus OC43 (taxid:31631)	Human coronavirus NL63 (taxid:277944)
Human coronavirus 229E (taxid:11137)	MERS coronavirus* (taxid:1335626)	Human Influenza A Virus (taxid:11320)
Influenza B virus (taxid:11520)	Human adenovirus 1* (taxid:10533)	Adenovirus 4* (taxid:28280)
Adenovirus 7* (taxid:10519)	Cytomegalovirus* (taxid:10358)	Human enterovirus EV68* (taxid:42789)
Epstein Barr virus* (taxid:10376)	Human parainfluenza 1 virus (taxid:12730)	Human parainfluenza 2 virus (taxid:1979160)
Human parainfluenza 3 virus (taxid:11216)	Human parainfluenza 4a virus (taxid:11224)	Human parainfluenza 4b virus (taxid:11226)
Measles morbillivirus* (taxid:11234)	Human Metapneumovirus (taxid:162145)	Mumps rubulavirus* (taxid:1979165)
Human respiratory syncytial virus (taxid:11250)	Rhinovirus (taxid:12059)	

Bacteria		
Bordetella pertussis (taxid:520)	*Chlamydia pneumoniae* (taxid:83558)	*Corynebacterium** (taxid:1716)
*Escherichia coli** (taxid:562)	*Hemophilus influenza** (taxid:727)	*Lactobacillus** (taxid:1578)
*Legionella pneumophila** (taxid:446)	*Moraxella catarrhalis** (taxid:480)	*Mycobacterium tuberculosis complex** (taxid:77643)
*Mycoplasma pneumoniae** (taxid:2104)	*Neisseria meningitides** (taxid:487)	*Neisseria** (taxid:482)
Pseudomonas aeruginosa group (taxid:136841)	*Staphylococcus aureus** (taxid:1280)	*Staphylococcus epidermidis* (taxid:1282)
*Streptococcus pneumoniae** (taxid:1313)	*Streptococcus pyogenes* (taxid:1314)	*Streptococcus salivarius** (taxid:1304)
*Pneumocystis jirovecii** (taxid:42068)	*Candida albicans* (taxid:5476)	

*BLASTn for homological analysis only

16. CLINICAL STUDY

The performance of the IntelliPlex SARS-CoV-2 Detection Kit was evaluated using contrived clinical nasopharyngeal (NP) swab specimens. Samples were prepared by spiking target RNA at different concentration into confirmed negative nasopharyngeal (NP) swabs in viral transport media (VTM). Negative NP swab samples were also tested.

The results are shown below. Clinical sensitivity = True positive/(true positive + false negative)* 100 % =97.5 % Clinical specificity = True negative/(true negative + false positive)* 100 % =100 %

Table 4. Performance Evaluation

		Confirmed Specimens	
		Positive*	Negative
SARS-CoV-2 Detection Kit	Positive	39	0
	Negative	1	30
	Total	40	30

***The test was done using contrived positive specimen (at ~ 1 x LoD = 140 copies/mL)

17. TROUBLESHOOTING

The troubleshooting listed below addresses possible problem causes and solutions provided during assay procedures.

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.
Positive Control Fail / Negative Control Fail	1. No POS or NEG Control added. 2. RNase contamination. 3. Assay did not work. 4. Cross-contamination between samples. 5. Wrong PC/NC wells chosen.	1. Ensure Controls are added. Ensure proper reconstitution of POS and NEG control as described. 2. Ensure all operating procedures are followed correctly and work environment is free of RNase. 3. Make sure all the assay procedures are followed correctly. 4. Clean all surfaces and equipment. Operate pre-PCR and post-PCR in the dedicated area and separate the equipment for use. 5. Choose the correct PC/NC wells and repeat reading.
πCode MicroDiscs Count / πCode MicroDiscs Combination Fail	1. πCode MicroDiscs are not properly dispersed in the well. 2. Not enough πCode MicroDiscs added to well. 3. Microbes exist in Wash buffers. 4. Instruments error or malfunction.	1. Re-disperse the microplate using IntelliPlex 1000 Processor, and repeat reading. 2. Ensure πCode MicroDiscs are well-mixed with proper amount added. 3. Use freshly prepared wash buffer and ddH_2O for hybridization to reduce πCode MicroDiscs loss rate. 4. Contact PlexBio Customer Service.
SA-PE Monitor Control Fail	1. No SA-PE was added or insufficient SA-PE solution for dispensing. 2. SA-PE solution deactivated. 3. Incorrect tested lanes of microplate selected for SA-PE solution dispensing.	1. Make sure all the assay procedures are followed correctly. Calculate sufficient SA-PE solution volume for dispensing. Repeat test. 2. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date. 3. Repeat assay and make sure lanes selected correctly.
Blank Control Fail	1. Wrong hybridization conditions. 2. Residues of SA-PE solution in wells after hybridization. 3. PlexBio 100 Fluorescent Analyzer is not calibrated. 4. Markings on plates.	1. Ensure correct hybridization program is selected. 2. Ensure all buffers (Wash buffer and ddH_2O) on IntelliPlex 1000 Processor are fresh-made and sufficient for washing procedures. 3. Perform calibration on PlexBio 100 Fluorescent Analyzer. 4. Do not make any marking on the plate.

Problem	Possible Cause	Recommendations
Reference Gene Control / Extraction Control Fail	1. RNA purification failed or PCR inhibitors existed.	1. Follow instructions of sample extraction carefully. Ensure required temperature ranges and centrifugation needs are complied. Ensure complete removal of ethanol.
	2. PCR procedures are not performed correctly.	2. Make sure all PCR procedures are followed correctly. Do not to use expired materials or mixed lots of reagents. Ensure storage conditions are correct.
	3. RNase contamination.	3. Ensure all the operating procedures are followed correctly. Ensure work environment is free of RNase.
	4. Hybridization did not work.	4. Make sure all the assay procedures are followed correctly. Ensure samples are freshly heat-denatured.

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
CE	European Union Conformity	EC REP	European Authorized Representative

Notice to User

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