COVID-19 RT-PCR PNA KIT

For *in vitro* diagnostic use only

For use under US FDA Emergency Use Authorization (EUA) only

Rx Only

Instructions for Use

Ver. 3.2

DIGITAL PCR FOR LIFE

bioTNS

CE IVD ISO 13485:2016

BioTNS Co., Ltd.

Indications of Medical Devices Act

1. Product Category: In vitro diagnostic Reagent for Infectious Agents-Virus
2. Product Name: COVID-19 RT-PCR PNA KIT
3. Product Catalogue Number: TD1100
4. Purpose of use: See 1. Intended use part in this User Guide

Warranty and Responsibility

All products of BioTNS Co., Ltd. are tested under strict quality management processes.
BioTNS Co., Ltd guarantees to ensure the quality of the product during warranty period, which written as until Expired Date.
If any problems relating to the quality of the product are found, please contact the headquarters immediately.

Quality Control System

All aspects of the quality management system, product creation, quality assurance, and supplier qualifications are certified to ISO 13485.

Inquiries and customer service (A/S)

Send us an e-mail (biotns@biotns.com) or call us at +82-42-861-2223 to inquire about the product. Additionally, US base phone number +1-770-891-3518 is also available for US users.

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KIT description

1. Intended Use

The COVID-19 RT-PCR Peptide Nucleic Acid (PNA) Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, bronchoalveolar lavage (BAL), and nasopharyngeal wash/aspirates or nasal aspirates 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 the 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 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 COVID-19 RT-PCR PNA Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The COVID-19 RT-PCR PNA Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

1. Intended Use

Special Conditions for Use Statements
-For Emergency Use Authorization (EUA) only
-For prescription use only
-For in vitro diagnostic use only

Special Instrument Requirements
- The COVID-19 RT-PCR PNA Kit is to be used with the CFX 96 Real-time PCR detection system (Bio-rad) or Applied Biosystems 7500/7500 Fast thermocycler instruments. Assay results are analyzed with the Bio-Rad CFX software, such as Bio-Rad CFX Maestro or Bio-Rad CFX Manager 3.0 or 3.1, or the 7500 software v2.3.

2. Product Overview

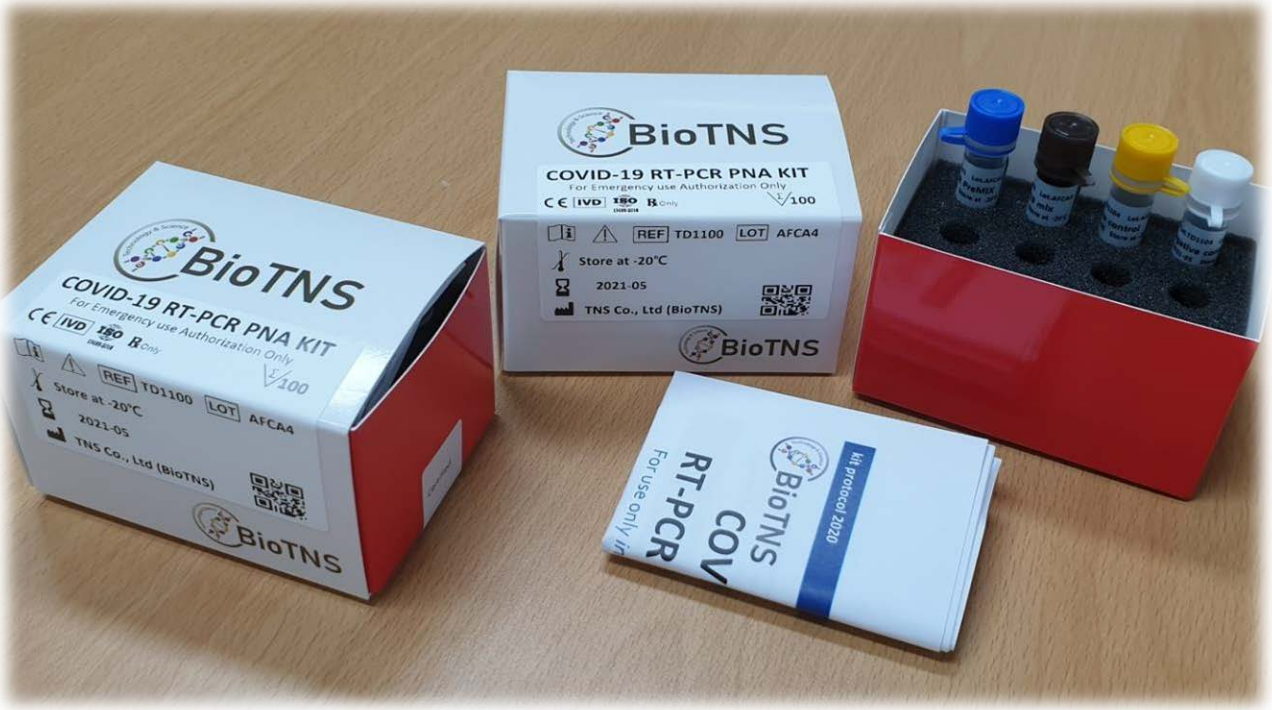
The COVID-19 RT-PCR PNA kit utilizes a Peptide Nucleic Acid (PNA) as a reusable fluorescence hybridization probe for real-time PCR. Peptide nucleic acids are artificially synthesized oligomers in which nucleic acid bases are attached to a neutrally charged backbone consisting of (repeating N-(2-aminoethyl)-glycine units) that form peptide bonds. PNAs exhibit stronger binding to their complementary sequences than a DNA oligomer would and cannot be degraded by exonuclease activity of enzymes such as DNA polymerase.

The kit targets two specific genomic regions of SARS-CoV-2 in a multiplex reaction: the RNA-dependent RNA Polymerase (RdRP) gene and the Nucleocapsid (N) gene. The PNA probes for the detection of SARS-CoV-2 are labeled with FAM (N gene) and HEX (RdRP) fluorescent dyes. Individual target amplification results are identified with different colors and thus able to be independently analyzed.

This kit also includes an internal control which amplifies the Human acidic ribosomal protein (HuPO) gene. Successful amplification of HuPO confirms correct RNA transcription to cDNA and subsequent PCR amplification. The PNA probe for the HuPO gene is labeled with a Cy5 fluorophore.

3. Product components

The COVID-19 RT-PCR PNA kit is composed of the components listed in the table below:



<Table 1> Information of KIT component

Cat. No	Component	Description	Cap/Tube	Quantity
TD1101	2X RT qPCR PreMix	Reverse transcriptase Taq polymerase PCR buffer	Clear tube / Blue cap ●	1000㎕/vial X 1ea
TD1102	COVID-19 mix	Primer, Probe mixture for SARS-CoV-2 and IC(HuPO)	Amber tube	500㎕/vial X 1ea
TD1103	Positive control	Template for SARS- CoV-2 and IC	Clear tube / Yellow cap ●	150㎕/vial X 1ea
TD1104	Negative control	Nuclease free Distilled Water	Clear tube / White cap ○	150㎕/vial X 1ea

4. Storage and Handling Requirements

1. The COVID-19 RT-PCR PNA Kit should be shipped and stored at -20℃.
   (Both un-opened and in-use product)

2. The component of COVID-19 mix should be stored away from light.
   COVID-19 mix contains light-sensitive fluorescent probes and exposure to light could reduce assay performance.

3. Kit materials are stable until the expiration date printed on the outer packaging when following proper storage requirement.
   Expiration date is a year later from manufactured date.
   But to ensure accurate results, reagents should be used as soon as possible once opened.

4. Excessive freeze-thawing of kit components may lead to inaccurate results.

5. Before each use, vortex thawed reagents for 10 seconds and spin down to collect at the bottom of the tube.

6. Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.

5. Additionally Required Materials and Equipment

The COVID-19 RT-PCR PNA Kit does not include sample collection and preservation instruments and buffers, RNA extraction reagents and Real-time PCR detection systems.
Following components are additionally required but not included with the kit. All these components are consumable.

- Disposable latex gloves and laboratory gowns
- Sterilized pipette tips (10㎕, 200㎕ and 1000㎕)
- Pipettes (10㎕, 200㎕ and 1000㎕)
- Sterilized (DNase, RNase free) microcentrifuge tube (1.5ml) and rack
- Nucleic acid extraction kit
  RNeasy Mini kit (Qiagen, Cat No. 74104 or 74106)
- Vortex mixer
- Desktop microcentrifuge
- DNA, RNAse decontamination agent or 70% Ethyl alcohol
- Bio-Rad CFX-96 real-time PCR detection system with C1000 Thermal cycler and software CFX Manager ver.3.0 or ver 3.1 or Maestro
  (Or ABI 7500/7500FAST system and software)
- 8 well PCR strips tube(White) and optical cap
- 96 well PCR plate (White) and sealing film for Real-time PCR equipment
- Biohazard waste container

6. Warnings and Precautions

- For emergency use only.
- For in vitro diagnostic use only (IVD).
- For Prescription Use Only (Rx).
- The COVID-19 RT-PCR PNA Kit has not been FDA cleared or approved; but 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.
- The COVID-19 RT-PCR PNA Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of the COVID-19 RT-PCR PNA Kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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 declaration is terminated or the authorization is revoked sooner.
- Use under the guidance of physicians and specialists.
- Please read the instructions for use carefully before testing.
- Store all Kit contents at -20℃ away from UV and sunlight.
- Avoid repeated freeze-thawing of un-opened Kit.
- Efficiency of reagents may be decreased with prolonged exposure to room temperature or light.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Improper specimen collection, transfer, storage, and processing may cause erroneous test results.

6. Warnings and Precautions

- Nucleic acid extraction should be performed as soon as possible after specimen collection to avoid viral nucleic acid degradation; if it cannot be performed as soon as possible, it should be stored in accordance with appropriate specimen collection, handling, and storage guidelines.

- As this test involves extraction of viral RNA and PCR amplification, care should be taken to avoid contamination of the amplification reaction mixture of the kit. Regular monitoring of laboratory contamination is recommended.

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.

- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

- Thoroughly clean up and disinfect all laboratory work surfaces with a freshly prepared solution with 70% ethyl alcohol or DNA/RNase decontamination agents.

7. Specimen Collection, Handling and Storage

Inappropriate specimen collection, storage, and transport may yield false test results. Appropriate training to collect and handle specimens is highly recommended due to the importance of specimen quality.

Collecting specimens

Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019Novel Coronavirus (SARS-CoV-2). (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Follow specimen collection device manufacturer's instructions for proper collection methods.

Swab specimens should be collected using only swabs with a synthetic tip, and an aluminum or plastic shaft. Calcium alginate swabs are not acceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3mL of viral transport media.

Wash/Aspirate samples - Use non-bacteriostatic saline (pH 7.0) to collect specimen and immediately place into sterile transport tube.

Bronchoalveolar lavage(BAL) - Collect 2-3mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including people admitted to the hospital and/ or fatal cases.

Shipping

Specimens must be packaged and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Store specimens at 2~8℃ and ship to the lab on ice pack. If a specimen is frozen at -70℃, ship to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

Criteria of rejection

The following specimens will be rejected prior to testing:
1) Specimens without sufficient volume for the test. (less than 1mL)
2) Specimens not stored proper conditions, as recommended in CDC guidelines.

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Technology & Process

1. Description of test step and technology used in Kit

The COVID-19 RT-PCR PNA Kit uses RNA isolated and purified from upper or lower respiratory specimens as template for PCR reaction.

This kit does not include reagents for the extraction of viral RNA. Nucleic acid is extracted using commercially available RNA preparation kit, RNeasy mini kit from Qiagen.

The 5$\mu\ell$ of purified nucleic acid is reverse transcribed into cDNA and amplified in the same reaction tube.

During amplification, the PNA probe anneals to specific target sequences located in the amplicon to generate a fluorescent signal.

In the process, the probe anneals to a specific target sequence located in the product of the Reverse primer since it more easily binds to single DNA strands than double strands.

The COVID-19 RT-PCR PNA Kit is a multiplex test that includes all assay primers (including the internal control) pre-mixed in the tube labeled COVID-19 mix.

2. Control materials to be Used with Kit

A Negative Control (NC):
contains nuclease free water intended to evaluate cross contamination of the kit, supplements, reagents and PCR instrument used in the test.
Negative control should be run using 5$\mu\ell$ in one well per test.

A Positive Control (PC):
contains artificially synthesized RNA of SARS-CoV-2 RdRP and N genes which is intended to evaluate analytical and clinical performance of the assay.
Also, the PC contains detectable short regions from the human acidic ribosomal protein (HuPO) gene that is also intended to evaluate performance reliability of the assay.

The positive control should be run using 5$\mu\ell$ in one well per test.

Internal Control (IC):
The Reaction Mixture tube of the kit, named COVID-19 mix, includes a primer set and probe to detect a specific region of Human acidic ribosomal protein (HuPO) gene. The internal control is intended to evaluate the RNA extraction process of clinical specimens.

Both Positive Control and Negative Control should be used directly with the test without prior dilution.

3. Assay Procedure

1. RNA extraction from clinical specimens

The COVID-19 RT-PCR PNA Kit does not include viral RNA extraction reagents. The RNeasy Mini kit (Qiagen, Cat No. 74104 or 74106) has been validated with the COVID-19 RT-PCR PNA Kit. The extraction kit requires 200 $\mu\ell$ sample input (both upper and lower respiratory tract specimens) with 350 $\mu\ell$ of Buffer RLT and finally yields 40$\mu\ell$ of purified nucleic acid eluent. Following the extraction, RNA should be used immediately or stored at -70~-20C (for up to 1 month) for use later.

2. Reaction master mix and Assay set up

*Note
- Plate set-up can be configured differently and modified with the number of specimens.
- Negative and Positive control must be included in each run.

1) Prepare reaction master mix in separate area (Assay preparation area) from nucleic acid handling.
   (1) Clean and decontaminate using 70% ethyl alcohol or commercially used decontamination reagents all work surfaces, equipment as well as small supplements e.g. pipette, vortex, micro centrifuge, prior to use to minimize the risk of nucleic acid cross-contamination.
   (2) Place enzyme mix on ice until thawed. Other reagents can be thawed at room temperature. Keep all reagents on ice once thawed during the whole test procedure.
   (3) Vortex vigorously for 10 sec and spin down all reagents before use.
   (4) Determine the number of reactions (N) to set up the assay.
   It is necessary to make extra reaction mix for PC, NC and for possible pipetting error. To allow for pipetting error, it is recommended to make the reaction master mix for TWO additional reactions.
   (5) Prepare the reaction master mix in a 1.5 mL microcentrifuge tube according to the following Table.
   A single reaction well requires 10$\mu\ell$ of 2X RT-qPCR PreMix and 5$\mu\ell$ of COVID-19 Mix for a total volume of 15$\mu\ell$ without added RNA sample, as described in the table below.

3. Assay Procedure

<Table 2> Composition of Master mix for reaction

Component	Amounts for 1test	Amounts for 8(+2) test	Amounts for 16(+2) test
2X RT-qPCR PreMix	10㎕	100㎕	180㎕
COVID-19 mix	5㎕	50㎕	90㎕
Total (Without RNA sample)	15㎕	150㎕	270㎕

(6) Vortex the prepared master mix for 10 sec and centrifuge briefly to collect contents at the bottom of the tube and place the tube in a cold rack.
(7) Set up white 96-well PCR plate or white 8-well PCR strips based on the number of test samples.
(8) Dispense 15㎕ of master mix into each well of white 96-well PCR plate or white 8-well PCR strips.
(9) Pipette 5㎕ of Negative control (NC) contains Nuclease free water, into NC sample well.

3. Assay Procedure

2) Nucleic acid template addition

*Attention
- Always change pipette tips in-between patient sample handling and after pipetting each component.
- Add the Positive Control (PC) in PCR plate last, to avoid the contamination. Positive control (PC) contains high concentration of viral template that tends to be contaminated easily.

*Change gloves often to avoid cross contamination between samples and control reagents.

(1) Gently vortex nucleic acid sample tubes for approximately 10 sec and spin down the tubes to collect contents at the bottom of the tubes. Always keep the sample tubes on ice or in a cold block.
(2) Dispense 5㎕ of nucleic acid samples into the 96 well PCR plate or 8-strip PCR tube containing the aliquoted reaction master mix.
(3) Carefully pipette 5㎕ of Positive control (PC) into a PCR plate well last.
(4) Seal the PCR plate or 8-strip tube with sealing film or cap strip. Ensure the sealing film is completely absorbed to the plate by using a roller.
(5) Optionally, spin down briefly using a micro plate centrifuge to collect the contents at the bottom of the well and remove extra air bubbles. It is recommended to centrifuge for 30 sec at 500 x g, 4℃.

3. Assay Procedure

3) Set up Real-time PCR run

*Note
- The thermocycler protocol described below is appropriate for either the Bio-Rad CFX96 instrument or the ABI7500/7500 Fast instruments.

- The run protocol and fluorescence channels for the targets are shown in Tables 3 and 4.

<Table 3> RT-PCR Thermocycling Condition (Run protocol)

Step	Temperature	Time	Repeat
cDNA synthesis	55℃	30min	1cycle
Pre-denaturation	95℃	10min	1cycle
Denaturation	95℃	30sec	45cycles
*Annealing	58℃	30sec	
Extension	72℃	30sec	

* Fluorescence signal is collected after each annealing step

<Table 4> Fluorescence Channel for the targets

Fluorescence signal	Target
FAM	N gene of SARS-CoV-2
HEX	RdRP gene of SARS-CoV-2
Cy5	HuPO gene of Human RNA as Internal control

**** Caution**
When using the ABI 7500/7500FAST instrument, set reference dye to 'None'.

3. Assay Procedure

CFX96 and Software Operation

- Case.1. New experiment
Follow the instructions below to set up a new experiment.

1) Turn on a computer and CFX 96.
2) Display the 96-well thermal block.
3) Place the 96 well plate prepared in previous step.
4) Run the CFX Manager software on the computer connected to the CFX96.
5) Go to File → New → Protocol → input the run information as shown in Table1.
   → Set the sample volume to 20㎕.
6) Go to Plate → Edit Selected → Set Fluorophores → Select fluorescence channel FAM, HEX and Cy5.
7) Specify the Positive control well, select "Positive Control" from sample type, and input 3 detection fluorescence in the load (Select FAM, HEX and Cy5).
8) Specify the Negative control well, select "Negative Control" from sample type, and input 3 detection fluorescence in the load (Select FAM, HEX and Cy5).
9) Wells with clinical specimens should be specified as Unknown, and input 3 detection fluorescence in the load (Select FAM, HEX and Cy5).
10) Go to Setting → plate type → Select BR white.
11) Go to Start Run → Select Block Name (PCR instrument) to use.
12) Click Close Lid and Click Start Run.

- Case.2. Repeat run
If you have a previous run file, you can re-use.

1) Double click on a previous run file.
2) Select File → Repeat Run.
3) Go to Plate tab → set Control and Sample information → Start Run.

* The fluorescence channel, plate type, and volume are already selected with previous run.

4. Interpretation of Results

The data is analyzed with analysis program of PCR instrument manufacturer.

The Ct value of the KIT is displayed in the analysis program and a Ct value ≤40 is reported as a positive result.

It is recommended that the threshold value is set to 20 RFU.

Manual adjustment of the threshold cutoff may be required if:

(1) low positive samples close to the threshold are observed or
(2) low positive and high positive samples are on the same plate.

4. Interpretation of Results

1) Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

- Negative Control:
  All assay targets including the RdRP gene, N gene, and HuPO IC must not be detected (Ct value >40).

- Positive Control
  All assay targets including the RdRP gene, N gene, and HuPO IC must be detected (Ct value ≤40).

- Internal Control (IC)
  The internal control (HuPO gene) must be detected (Ct ≤40) to successfully report a negative result. If the internal control result is negative and SARS-CoV-2 specific targets are also negative, the result is invalid and testing should be repeated as described below. If any of the SARS-CoV-2 specific targets are positive, the internal control result does not need to be detected for the assay result to be considered valid.

- Positive and Negative Controls should meet the requirements listed the below table to ensure valid results.

<Table 5> Quality Control Valid Results

Control	Results (Ct Value)		
	N gene (FAM)	RdRP gene (HEX)	HuPO gene (Cy5)
Negative	-	-	-
Positive	≤40	≤40	≤40

4. Interpretation of Results

2) Results interpretation

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. If the values of the controls are conclusive, users should refer to the interpretation algorithm table below to determine the infection status of the patient specimen.

<Table 6> Result Interpretation

Results			interpretation
N gene (FAM)	RdRP gene (HEX)	HuPO (IC) (Cy5)	
Ct >40	Ct >40	Ct ≤40	Negative (Absence of SARS-CoV-2 RNA)
Ct ≤40	Ct ≤40	Any Result	Positive (Presence of SARS-CoV-2 RNA)
Ct ≤40	Ct >40	Any Result	Positive (Presence of SARS-CoV-2 RNA) -Result is suggestive of (1) A sample at concentrations near or below the limit of detection of the test (2) A mutation in one of the target regions (3) infection with some other sarbecovirus currently unknown to infect humans
Ct >40	Ct ≤40	Any Result	
Ct >40	Ct >40	Ct >40	Invalid -Re-test after confirmation of PCR mixture preparation step, PCR protocol, and Kit storage conditions. -If the result is still invalid, repeat extraction and testing of the specimen.

4. Interpretation of Results

All clinical samples should exhibit fluorescence growth curves in the Internal control (IC), the Human acidic ribosomal protein (HuPO) gene, that cross the threshold line at or before Ct 40 to indicate the presence of the HuPO gene. Failure to detect HuPO in any clinical specimens may indicate:

(1) Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
(2) Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
(3) Improper assay set up and execution.
(4) Reagent or equipment malfunction.

If the IC reaction does not produce a positive result for human clinical specimens, interpret as follows:

(1) If either of the SARS-CoV-2 targets (N gene and RdRP gene) is positive even in the absence of a positive IC result, the SARS-CoV-2 target result should be considered valid. It is possible, that some samples may fail to exhibit HuPO amplification curves due to low cell numbers in the original clinical sample.

(2) If all SARS-CoV-2 markers (N gene and RdRP gene) and IC (Human acidic ribosomal protein-HuPO) are negative for the specimen, the result should be considered invalid for the specimen. If residual RNA extract remains, repeat the test with this material. If the result remains invalid, repeat the extraction procedure with residual specimen and repeat the test. If all markers remain negative after re-test, report the results as invalid and re-collect patient sample.

5. Limitations

The use of this assay as an in vitro diagnostic under the 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 the requirements to perform high complexity tests.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

Specimens must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may disturb the ability of the assay to detect the target genes.

Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:
- o Improper specimen collection
- o Degradation of the viral RNA during shipping/storage
- o Using unauthorized extraction or assay reagents
- o The presence of RT-PCR inhibitors
- o Mutation in the SARS-CoV-2 virus
- o Failure to follow instructions for use

False-positive results may arise from:
- o Cross contamination during specimen handling or preparation
- o Cross contamination between patient samples
- o Specimen mix-up
- o RNA contamination during product handling

Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

A positive result indicates the detection of nucleic acid from SARS-CoV-2.

Nucleic acid may persist even after the virus is no longer viable.

5. Limitations

The performance of the COVID-19 RT-PCR PNA Kit was established using nasopharyngeal swab samples. Oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, bronchoalveolar lavage (BAL), and nasopharyngeal wash/aspirates or nasal aspirates are also considered acceptable specimen types for use with the COVID-19 RT-PCR PNA Kit but performance has not been established.

The COVID-19 RT-PCR PNA Kit can be used only with the specimens listed in the Intended Use statement. Other specimen types have not been evaluated and should not be tested with this assay.

The instrument and assay procedures reduce the risk of contamination by amplification product.

However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

As with any molecular test, mutations within the target regions of COVID-19 RT-PCR PNA Kit assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.

Laboratories are required to report all results to the appropriate public health authorities.

The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

6. Conditions of Authorization for the Laboratory

The COVID-19 RT-PCR Peptide Nucleic Acid (PNA) 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 FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizationsmedical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the COVID-19 RT-PCR PNA Kit, the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using the COVID-19 RT-PCR PNA Kit must 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 the COVID-19 RT-PCR PNA Kit must use the COVID-19 RT-PCR PNA Kit 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 the COVID-19 RT-PCR PNA KIT are not permitted.

c) Authorized laboratories that receive the COVID-19 RT-PCR PNA Kit must notify the relevant public health authorities of their intent to run the COVID-19 RT-PCR PNA Kit prior to initiating testing.

d) Authorized laboratories using the COVID-19 RT-PCR PNA Kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

e) Authorized laboratories must collect information on the performance of the COVID-19 RT-PCR PNA Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and BioTNS Co., Ltd. (via email biotns@biotns.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the COVID-19 RTPCR PNA Kit of which they become aware.

f) All laboratory personnel using the COVID-19 RT-PCR PNA Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the COVID-19 RT-PCR PNA Kit in accordance with the authorized labeling.

g) BioTNS Co., Ltd, authorized distributors, and authorized laboratories using the COVID-19 RT-PCR PNA Kit must 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.”

03

Analytical Performance

1. Limit of Detection (Analytical Sensitivity)

Limit of detection (LoD) of the Kit is defined as the lowest detectable concentration of SARS-CoV-2 RNA at which greater than or equal to 95% of all replicates test positive (for example 19/20 replicates are positive).

The LoD of the COVID-19 RT-PCR PNA Kit was determined using dilutions of SARS-CoV-2 positive clinical nasopharyngeal (NP) swab specimens. Viral RNA was quantitated in patient samples by comparison to a standard curve prepared from serial dilutions of SARS-CoV-2 IVT RNA transcript in NP swab matrix.

These analyses were performed on the Bio-Rad CFX96 instrument using a Qiagen RNeasy mini extraction kit.

A preliminary estimate of the LoD for both the N and RdRP assay targets was obtained by evaluating two-fold serial dilutions of a quantitated patient specimen in triplicate.

The lowest analyte concentration for which 3/3 replicates was positive was 3.715 copies/$\mu\ell$ for the N assay target and 2.524 copies/$\mu\ell$ for the RdRP assay target.

The estimated LoD was further confirmed by evaluating an additional 20 replicates of diluted patient specimen at the estimated LoD for each individual assay target. Both N and RdRP assay targets exhibited 100% reactivity at the estimated LoD as shown in the tables below.

1. Limit of Detection (Analytical Sensitivity)

<Table 7> Triplex test for determining N gene's LoD for Clinical specimens

Dilution Ratio	Quantitated Clinical NP Swab Specimen		
	14.86 copies/㎕		
1	30.41	30.24	30.18
2	31.31	31.13	31.50
4	32.20	32.87	32.37
8	N/A	31.94	30.81
LoD	**Dilution factor 4 = 3.715 copies/ ㎕**		

<Table 8> Final concentration of N gene's LoD of Clinical specimens test

1X LoD	1	2	3	4	5	6	7	8	9	10	Posi. Rate
Ct Value	32.58	32.43	32.04	31.83	31.53	31.51	31.49	31.46	31.41	31.35	
1X LoD	11	12	13	14	15	16	17	18	19	20	100% (20/ 20)
Ct Value	31.21	31.14	31.07	30.99	30.94	30.93	30.82	30.79	29.79	29.60	

<Table 9> Triplex test for determining RdRP gene's LoD for Clinical specimens

Dilution Ratio	Quantitated Clinical NP Swab Specimen		
	5.048 copies/㎕		
1	32.21	32.42	32.28
2	33.65	34.00	33.64
4	N/A	N/A	N/A
8	N/A	N/A	N/A
LoD	**Dilution factor 2 = 2.524copies/ ㎕**		

<Table 10> Final concentration of RdRP gene's LoD of Clinical specimens test

1X LoD	1	2	3	4	5	6	7	8	9	10	Posi. Rate
Ct Value	35.41	35.27	34.19	34.19	33.73	33.61	33.55	33.48	33.44	33.40	
1X LoD	11	12	13	14	15	16	17	18	19	20	100% (20/ 20)
Ct Value	33.38	33.10	33.10	33.08	32.90	32.80	32.73	32.57	32.21	31.96	

2. Limit of Detection - Instrument Comparison

In order to establish equivalent performance of the COVID-19 RT-PCR PNA Kit on the ABI 7500/7500 fast instrument, the same clinical specimens utilized in the original LoD study were further tested in serial two-fold dilutions near the LoD for each assay target. Both assay targets achieved 100% reactivity and demonstrate equivalent assay performance between these two thermocycler instruments.

<Table 11> 5 replicates test for determining N gene's LoD for Clinical specimens with ABI 7500/7500FAST

Dilution Ratio	1X LoD of N gene at CFX96				
	3.715 copies/㎕				
2X LoD	30.34	30.16	30.27	30.14	30.14
1X LoD	31.74	31.29	32.16	31.71	31.67
0.5X LoD	N/A	N/A	N/A	N/A	N/A
0.25X LoD	N/A	N/A	N/A	N/A	N/A
LoD	**1X = 3.715 copies/ ㎕**				

<Table 12> 5 replicates test for determining RdRP gene's LoD for Clinical specimens with ABI7500/7500FAST

Dilution Ratio	1X LoD of RdRP gene at CFX96				
	2.524 copies/㎕				
2X LoD	29.71	29.78	28.07	29.19	29.95
1X LoD	31.23	30.71	31.54	31.15	31.25
0.5X LoD	N/A	N/A	N/A	N/A	N/A
0.25X LoD	N/A	N/A	N/A	N/A	N/A
LoD	**1X = 2.524copies/ ㎕**				

3. Inclusivity (Analytical Reactivity)

The COVID-19 RT-PCR PNA KIT was evaluated using publicly available full and partial SARS-CoV-2 genome sequences. Sequences were downloaded from following Databases;
(1) NCBI(GenBank) : https://www.ncbi.nlm.nih.gov/genbank/
(2) GISAID : https://www.gisaid.org/

Sequence analysis was performed using the tool in CLC workbench 8.0 software. Primers and Probes used in the COVID-19 RT-PCR PNA kit were designed to target the RdRP gene and N gene of SARS-CoV-2.

In silico analysis results have shown that the primers and probe sequences of the COVID-19 RT-PCR PNA kit are able to detect all analyzed SARS-CoV-2 sequences in NCBI and in GISAID databases as of as of April 13, 2020 KST.

The primers and probe for RdRP gene of SARS-CoV-2 of the COVID-19 RT-PCR PNA KIT exhibit 100% identity to all sequences of the RdRP gene, including 98 NCBI (GENBANK) sequences and 5,125 full genome sequences in the GISAID database.

The primers and probe for N gene of SARS-CoV-2 of the COVID-19 RT-PCR PNA KIT exhibit 100% identity to all sequences of the N gene, including 385 NCBI (GENBANK) sequences and 5,125 full genome sequences in the GISAID database.

No mismatches were observed between the Kit primers and probe sequences and SARS-CoV-2 sequences.

4. Cross-reactivity (Analytical Specificity)

To evaluate analytical specificity of the COVID-19 RT-PCR PNA Kit both wet testing and *in silico* analysis was performed against organisms commonly found in in upper respiratory specimens (such NPS, OPS and nasal swabs) or BAL specimens or from organisms that can cause similar clinical symptoms.

<Table 13> List of Organisms to be analyzed *in silico* and by Wet Testing

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius

4. Cross-reactivity (Analytical Specificity)

1) *In-silico* Analysis

In silico BLASTn analysis with queries of the COVID-19 RT-PCR PNA Kit primers and probes was performed against public domain nucleotide sequences contained in the NCBI database.

No significant homology was observed for the N primer and probe sequences across all organisms analyzed. The RdRP forward primer exhibited 92% homology with SARS coronavirus and the RdRP probe shared 83% homology with SARS coronavirus. The RdRP reverse primer exhibited no significant homology to the SARS sequence. Since the SARS coronavirus is not currently circulating and significant homology is not observed for all assay components, the risk of false positive results is low.

In conclusion, the COVID-19 RT-PCR PNA KIT primers and probes have high specificity for SARS-CoV-2 target genes.

4. Cross-reactivity (Analytical Specificity)

2)Wet Testing

The 18 organisms and viruses, listed in Table 14, were wet-tested for cross-reactivity with the COVID-19 RT-PCR PNA Kit.

All bacterial and viral pathogens were spiked in triplicate into negative NP swab matrix at concentrations corresponding to ≥10^6 copies/μL and evaluated by the COVID-19 RT-PCR PNA Kit.

No cross-reactivity was observed for the organisms and viruses listed Table 14.

<Table 14> Cross-reactivity Wet Testing Target and Results

Organisms	Wet Testing Result		
	First	Second	Third
Human coronavirus 229E	Negative	Negative	Negative
Human coronavirus OC43	Negative	Negative	Negative
Human coronavirus NL63	Negative	Negative	Negative
Adenovirus subtype B	Negative	Negative	Negative
Adenovirus subtype C	Negative	Negative	Negative
Human Metapneumovirus (hMPV)	Negative	Negative	Negative
Human parainfluenza 1	Negative	Negative	Negative
Human parainfluenza 2	Negative	Negative	Negative
Human parainfluenza 3	Negative	Negative	Negative
Human parainfluenza 4	Negative	Negative	Negative
Influenza A	Negative	Negative	Negative
Influenza B	Negative	Negative	Negative
Human Respiratory syncytial virus A	Negative	Negative	Negative
Human Respiratory syncytial virus B	Negative	Negative	Negative
Rhinovirus A	Negative	Negative	Negative
Rhinovirus B	Negative	Negative	Negative
Bordetella pertussis	Negative	Negative	Negative
Mycoplasma pneumoniae	Negative	Negative	Negative

5. Clinical Evaluation

For the clinical evaluation study, testing was performed on left-over archived nasopharyngeal (NP) swab samples from symptomatic patients suspected of COVID-19 infection. Specifically, clinical NP swab specimens that were previously tested using the EUA authorized Seegene Allplex 2019-nCoV Assay were tested using the COVID-19 RT-PCR PNA Kit. A total of 33 positive samples and 32 negative samples, as determined by the comparator method, were utilized in this study.

The results from testing report that 100% (33/33) positive and 100% (32/32) negative agreement for all clinical specimens tested.

These results are acceptable and support to use of the COVID-19 RT-PCR PNA Kit for testing clinical specimens.

The details of test results are shown to below table.

<Table 15> Clinical Evaluation Comparator Test Results

		Seegene Comparator Results	
		Positive	Negative
COVID-19 RT-PCR PNA Kit	Positive	33	0
	Negative	0	32

Positive percent agreement: 100% (33/33) 95% CI: 89.6-100%
Negative percent agreement: 100% (32/32) 95% CI: 89.3-100%

6. FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material(T1), blinded samples and a standard protocol provided by the FDA.

The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD.

The extraction method and instrument used were RNeasy mini kit from Qiagen and Biorad CFX96 real-time PCR system.

The results are summarized in Table 16.

<Table 16> Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP swab	5.4×10^5 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

04

Reference & Symbols

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2. Symbols

<Table 16 > Symbols used in the kit

Symbol	Meaning
	For prescription only
	For *in vitro* Diagnostic Use
	Catalog Number
	Lot Number
	Temperature Limit (Store temperature)
	Used by (Expired date)
	Operating instructions
	Caution
	Number of Test
	Manufacturer

BioTNS Co., Ltd.

COVID-19 RT-PCR PNA KIT

For *in vitro* diagnostic use only

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CE IVD ISO 13485:2016

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