BioTNS

TNS Co., Ltd.

COVID-19 RT-PCR PNA KIT

For use only *in vitro* diagnostic

Cat. TD1100

For Emergency Use Authorization Only

CE IVD ISO 13485:2016 Rx Only -20℃ Σ 100

• Intended use

The COVID-19 RT-PCR PNA KIT is an in vitro diagnostic product with real-time reverse transcription(RT)-Polymerase chain reaction (PCR) assay intended for the qualitative detection of nucleic acid form the SARS-CoV-2 known as SARS-2019-nCoV(N gene and RdRP gene), novel coronavirus in respiratory specimens such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate nasal swabs, bronchoalveolar lavage and sputum collected from individuals who are suspected of COVID-19 with presenting clinical signs or meeting COVID-19 epidemiological criteria.

The Kit specially using PNA(Peptide nucleic acid) probe, that false-positive is not produced oppositely which method using the TaqMan probe is possible to.

• Storage and Handling Requirements

- The KIT should be shipped and stored at -20℃ below.
- The component of COVID-19 mix should be stored away from light. COVID-19 mix contains fluorescent dye labelled probes which performance is affected by light that effects to Kit performance.
- Kit materials are stable until the expiration date printed on the outer packaging when following proper storage requirement. Officially, Expiration date is a year later from manufactured date. But It is recommended for accurate results, Used out the reagents as soon as possible once it opened.
- Excessive Freeze-thawing of kit components may lead to inaccurate results.
- Before each use, Vortex 10 sec strong and spin down briefly the reagents.

• Warnings and Precautions

- The KIT is for in *vitro* diagnostics use only or for prescription use only.
- All details follow guideline for wearing personal protect equipment and collecting and handling and shipping samples and transport samples to use this kit.
- DO use under the guidance of physicians and specialists.
- DO read the instructions for use carefully before testing.
- DO dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- DO NOT expose kit to UV.
- DO NOT repeat the freeze-thawing of the kit.
- DO NOT expose hazardous or biologically contaminated materials.
- DO NOT reuse disposable materials used in experiments.
- All clinical samples should be treated as infectious substance.

• Product components

The COVID-19 RT-PCR PNA KIT is designed to type of Master mix for user's convenience. A kit provide quantity for 100 test

Component	Quantity
2X RT qPCR PreMix	1000㎕/vial X 1ea (Blue cap ●)
COVID-19 mix	500㎕/vial X 1ea (Amber cap ●)
Positive control	150㎕/vial X 1ea (Yellow cap ●)
Negative control	150㎕/vial X 1ea (White cap ○)

• Protocol

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) or RNA extraction kit certificated to Viral RNA were available and following the manufacturer`s instructions. recommended specimens are Respiratory specimens which are self-collected or collected by healthcare provider.

2. Reaction master mix and Assay set up

1) Clean and decontaminate using 70% ethyl alcohol or commercially used decontamination reagents all work surfaces, equipment
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 for 10 sec strong and spin down all reagents before use
4) Determine the number of reactions (N) to set up the assay
5) Prepare the reaction master mix in 1.5 ㎖ microcentrifuge tube according to the following table
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 96 well PCR plate or 8-strips PCR tube as following amounts of needing test samples
8) Dispense 15 ㎕ of master mix into the wells of 96-well PCR plate or 8-strips PCR tube
9) Pipette 5 µL of Negative control(NC) contains Nuclease free water into NC sample well

Component	1 test	8(+2) tests	16(+2) tests	32(+2) tests
2X RT qPCR PreMix	10 ㎕	100 ㎕	180 ㎕	340 ㎕
COVID-19 Mix	5 ㎕	50 ㎕	90 ㎕	170 ㎕
Total (Without sample)	15 ㎕	150 ㎕	270 ㎕	510 ㎕

* Use at least one Positive control and Negative control per a test as experimental valid controls.
* Furthermore Total of Prepare the reaction master mix is with amounts of Determined the number of reactions (N) + TWO more at least.

3. Nucleic acid template addition

1) Gently vortex sample tubes for approximately 10 sec and spin down before use
2) Dispense nucleic acid samples (purified RNA samples) of 5 ㎕ 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 tube with sealing film or cap strip

4. Set up Real-time PCR run

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

1) Place the PCR tube or plate into the PCR instrument
2) Perform the test following PCR protocol table

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

* Collect fluorescence signal after each Annealing step done

Fluorescence signal	Target
FAM	N gene of SARS-CoV-2
HEX	RdRP gene of SARS-CoV-2
Cy5	HuPO gene of Human RNA(IPC)

* Caution: On ABI 7500/7500FAST, Select reference dye Not 'RoX' But 'None'

• Data Analysis

The data is analyzed with analysis program of PCR instrument manufacturer. The analysis Ct value of the KIT is displayed in the analysis program and the Cut off that Ct value ≤40 is the standard of Validity of test results. The Ct value is analyzed with Automatically analyzed threshold on program basically so no additionally setting is not need.

But the cases that (1) very low signal near to baseline noise signal or (2) very difference with signal the most high and low concentration samples, It is recommend that setting the proper threshold with including low positive signal and It have to be on upper site than baseline noise signal.

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
  Both RdRP(HEX) gene and N(FAM) gene of SARS-CoV-2 must not be detected, and the Ct value of IPC(Cy5) should be none.

- Positive control
  Both RdRP(HEX) gene and N(FAM) gene of SARS-CoV-2 must be detected, and the Ct value of IPC(Cy5) should be ≤40.

- Internal Positive Control (IPC)
  If the result for a specimen is SARS-CoV-2 RNA not detected, the Ct value of the IPC must be ≤40, otherwise the result of that specimen is inconclusive If the result for a specimen is SARS-CoV-2 RNA detected, the Ct value of the IPC is not required to be considered valid.

- Positive and Negative controls should meet the requirements listed the following table to ensure valid results.

- If negative and positive control results are not as described above, the test results of the entire batch are invalid.

Control	Results (Ct value)		
	N gene (FAM)	RdRP gene (HEX)	IPC (Cy5)
Negative	-	-	-
Positive	**≤40**	**≤40**	**≤40**

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, refer to the table below to determine the infection status, expectedly.

* The data is result of PNA(Peptide Nucleic Acid) Probes interaction with target cDNA sequence. PNA probe has very high affinity to DNA but very specific binding status depend on temperature change. And also it is not cleaved by enzymatically that This Kit does not produce false positive results.

Results (Ct value ≤40)			interpretation
N gene (FAM)	RdRP gene (HEX)	IPC (Cy5)	
-	-	+	Negative (Absence of SARS-CoV-2 RNA)
+	+	+/-	Positive (Presence of SARS-CoV-2 RNA)
+	-	+/-	Positive*1) (Presence of SARS-CoV-2 RNA)
-	+	+/-	
-	-	-	Invalid**2)

*1) Result is suggestive of
- A sample at concentrations near or below the limit of detection
- A mutation in one of the target regions
- Other factors

**2) Invalid test is followed as
- Re test after confirmation of PCR mixture preparation step and PCR protocol and Kit storage condition and validity.
- If available, Repeat test with same RNA extract.
- If the result is still invalid, a new specimen should be obtained.

• Special Instrument requirements

- CFX 96 Real-time PCR system (bio-rad)
- ABI 7500/7500FAST (Applied Biosystems)

• Technical Support

Please contact via e-mail(biotns@biotns.com) for User's Guide, general inquiry and technical supports.

BioTNS
TNS Co., Ltd.
5-6F, 19-12, Daehak-ro 76beonan-gil,
Yuseong-gu, Daejeon, Korea
TEL : +82.42.861.2223
FAX :+82.42.861.2229