NANO TECHNOLOGY ADD FOR INNOVATION

BNG® COVID-19 RT-qPCR Detection Kit

INSTRUCTIONS FOR USE

REF

IVD

RT-qPCR.018 - 100 Test

RT-qPCR.018500 - 500 Test

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BNG® COVID-19 RT-qPCR Detection Kit Instructions For Use

1. INTENDED USE

- **BNG® COVID-19 RT-qPCR Detection Kit** is a real-time RT-PCR test intended for the qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes, bronchoalveolar lavage and saliva specimens. This document describes the use of real-time RT-qPCR assays for the *in vitro* qualitative detection SARS-CoV-2 in respiratory specimens. The SARS-CoV-2 primer and probe sets are designed for the specific detection of SARS-CoV-2.
- **BNG® COVID-19 RT-qPCR Detection Kit** detects SARS-CoV-2 RNA in nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes, bronchoalveolar lavage and saliva samples during infection. Positive results indicate the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information must be considered to determine the actual patient infection status. Positive results do not exclude bacterial infection or co-infection with other viruses.

- Negative results do not exclude a SARS-CoV-2 infection and must 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 use of **BNG® COVID-19 RT-qPCR Detection Kit** is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- **BNG® COVID-19 RT-qPCR Detection Kit** is a multiplex kit. It can perform both human extraction and COVID-19 testing in the same well.
- **BNG® COVID-19 RT-qPCR Detection Kit** Real-Time PCR Kit is an *in vitro* nucleic acid amplification assay for qualitative detection of SARS-CoV-2 in respiratory specimens using quick molecular transport and isolation kits and usual isolation kits. Such as **BNG® Quick RNA Molecular Transport Isolation Kit, BNG® Quick RNA Isolation Kit,** and Quant Studio 5 Real-Time PCR Detection Systems, Rotor-Gene 3000/6000, Applied Biosystems 7500, BIO-RAD CFX96-IVD 7500 Fast System (ABI®), Agilent AriaMx Real Time PCR systems, LongGene Q2000A, StepOnePlus™ Real-Time PCR System, SLAN-96P and Bioneer-Exicycler-96
- The kits follow CDC's and WHO's latest detection guidelines.

2. PRODUCT DESCRIPTION

BNG® COVID-19 RT-qPCR Detection Kit is a real-time RT-qPCR based detection system for the 2019 Wuhan coronavirus **(SARS-CoV-2**, formerly **2019-nCoV).** SARS-CoV-2 is considered a novel human coronavirus that is genetically distinct from the common human coronaviruses (229E, NL63, OC43, HKU1), which cause seasonal acute respiratory illness. It is also genetically distinct from the newer human coronavirus, MERS-CoV and SARS-CoV.

The **BNG® COVID-19 RT-qPCR Detection Kit** is produced with a non-interfering blue colored visible follow-up dye in the kit solution to eliminate pipetting error. (Kit solution is colored (blue)).

BNG® COVID-19 RT-qPCR Detection Kit SARS-CoV-2: Detects the presence of 2 different highly specific gene sequences of N, Orf1ab, RdRp, S and E gene.

To confirm that the sample is positive for SARS-CoV-2, one of the two gene regions must test positive.

Additionally, a non-infectious positive control and a negative human extraction control are included. **Human Extraction Control (HEC)** is needed to ensure appropriate RNA extraction, purification and reverse transcription and all reagents involved in reaction. **Human Extraction Control (HEC)** included in the mix contains primers and probe for an endogenous human target extracted from the swab during the extraction step. We don't put an external DNA or RNA template as extraction control since we already get human target during extraction. The positive control is used to confirm functionality of the assays and overall PCR performance, the negative human extraction control is included to evaluate the quality of the RNA isolation independently from the SARS-CoV-2 assays.

2.1. REAL TIME PCR-BASED DETECTION OF SARS-CoV-2

The first step in the detection of SARS-CoV-2 is the conversion of viral RNA into cDNA. Afterwards, the target sequences unique for SARS-CoV-2 are specifically amplified with amplification monitored in real time through the use of fluorescently labelled probes: upon incorporation into the newly amplified DNA strands, the fluorophore (FAM™, HEX™) is released and an increase in fluorescence signal can be observed.

Due to the intrinsic mutation rate of coronaviruses, it is possible that mutations in the target sequence occur and accumulate over time. This can lead to false-negative results with a PCR-based detection approach.

The BNG® COVID-19 RT-qPCR Detection Kit addresses this issue by using 2 detection tests on 2 different target sequences to minimize the possibility of false negative results caused by an altered target sequence. The original target sequences for SARS-CoV-2 are included as a non-infectious target positive control (TPC) to check the integrity of the detection assays.

Samples tested positive should always be confirmed through complementary methods and additional analysis in an independent laboratory.

BNG® COVID-19 RT-qPCR Detection Kit is compatible with every qPCR with calibrated FAM™ and HEX/VIC/JOE channel. It has been formulated with a

reference dye compatible with a variety of qPCR instrument types, including those that do not use passive reference dyes and those using low and high concentration passive reference dye (ROX). (No additional components are needed to ensure compatibility with these devices).

2.2. MATERIALS PROVIDED

	Reagents	Quantity and Volume (100 tests)	Quantity and Volume (500 tests)
1	BNG One Step Reaction Mix	1 x 1500 µL	1 x 7500 µL
2	Negative Control(NTC)	1 x 500 µL	1 x 2500 µL
3	Target Positive Control(TPC)	1 x 500 µL	1 x 2500 µL

3. ADDITIONAL MATERIALS REQUIRED

- Suitable means & equipment for nucleic acid extraction
- Real-time PCR detection system equipped for FAM™ and HEX/VIC/ JOE detection
- Adjustable pipettes & fitting filtered pipette tips
- Appropriate personal protective equipment & workspaces for working with potentially infectious samples
- Surface decontaminants such as DNAZap™ (Life Technologies), DNA Away™ (Fisher Scientific), RNAse Away™ (Fisher Scientific), 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)
- Nuclease-free tubes / strips / plates to prepare dilutions, mastermixes etc.
- Nuclease-free tubes / strips / plates corresponding to the PCR device
- Suitable storage options for reagents and specimen (4^0C, -20^0C, -70^0C)

4. STORAGE

- Store all components at -20^0C and avoid repeated freeze and thaw cycles
- Protect the qPCR mastermixes from light as prolonged exposure can diminish the performance of the fluorophores.
- If the kit components have been damaged during transport, contact BNG Laboratories. Do not use as performance may be compromised.
- Keep reagents separate from sample material to avoid contamination.
- Do not use after the designated expiry date.

5. WARNINGS

- Kit; It should be stored away from nucleic acid sources and qPCR amplicons.
- The components in the kit should not be mixed with components with different lot numbers or with chemicals with the same name but from different manufacturers.
- Master stock reagents should be stored on the cold block during the PCR setup, if possible, and the PCR setup should be performed on the cold block if possible.
- The kit components should be mixed by gently shaking before use.
- The micropipettes used to pipette qPCR mixes and template nucleic acids must be separate.
- Mold nucleic acid and positive control tubes must be kept closed, except for liquid transfers.
- Cleanable surfaces of rooms, benches, and devices where analysis will be carried out should be regularly cleaned with 10% NaCIO, RNAse away and 70% Ethonol.
- Reaction tubes with completed qPCR should be disposed of before being opened in the laboratory.

6. PERFORMANCE CHARACTERISTICS

Clinical Performance

The overall **BNG® COVID-19 RT-qPCR Detection Kit** tests resulted in 277 true positives, 100 true negatives and 1 false negative. Sensitivity and specificity of the **BNG® COVID-19 RT-qPCR Detection Kit** are 99.6% and 100% respectively.

Patient Specimens (for all sample types)		Clinical test		
		Positive	**Negative**	**Total**
BNG® COVID-19 RT-qPCR Detection Kit	**Positive**	276	0	276
	Negative	1	100	101
	Total	277	100	377
Positive Percent Agreement		276/277= 99.6 %		
Negative Percent Agreement		100/100=100%		

Analytical sensitivity

Analytical sensitivity of **BNG® COVID-19 RT-qPCR Detection Kit** was analayzed using dilution series of control samples. Limit of Detection (LoD) value is 10cp/µL confidence ranges are summarized in Table 1.

Target Gene	Limit of Detection (copies/ µL)
N /Orf1ab/RdRp/S/E	10

Table 1: BNG® COVID-19 RT-qPCR Detection Kit - Limit of Detection (LoD) values

Diagnostic specificity

SARS-CoV-2 RNA negative clinical specimens were analyzed to determine the diagnostic specificity of **BNG® COVID-19 RT-qPCR Detection Kit**. 100 SARS-CoV-2 RNA negative clinical swab specimens were used. None of the 100 SARS-CoV-2 negative clinical specimens gave positive test result for SARS-CoV-2. Diagnostic specificity of **BNG® COVID-19 RT-qPCR Detection Kit** is 100 %. All of the internal control gave positive result.

Cross-Reactivity

In this study, the specificity of the assay was evaluated by testing 24 reference potential cross-reactive markers. **BNG® COVID-19 RT-qPCR Detection Kit** do not show any cross-reactivity with other potential cross-reactive markers given in the Table 2 below:

TARGET	RESULT	TARGET	RESULT
HCoV-HKU1	Negative	Respiratory sysncytical virus (A/B)	Negative
HCoV-OC43	Negative	Parainfluenza 1 virus	Negative
HCoV-NL63	Negative	Parainfluenza 2 virus	Negative
HCoV-229E	Negative	Parainfluenza 3 virus	Negative
MERS-CoV	Negative	Parainfluenza 4 virus	Negative
Influenza A(H1N1)	Negative	Human metapneumo virus	Negative
Influenza A(H3N2)	Negative	Adenovirus	Negative
Influenza A(untyped)	Negative	Human bocavirus	Negative
Influenza A(H5N1)	Negative	*Legionella* spp.	Negative
Influenza B (Victoria or Yamagata)	Negative	*Mycoplasma* spp.	Negative
Rhinovirus/enterovirus	Negative	HRV	Negative
MERS	Negative	TPC	Positive
HMPV	Negative	NTC	Negative

7. BIOSAFETY

- Wear appropriate personal protective equiment (e.g. gowns, powder-free gloves, eye protection) when working with clinical specimens.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
- For more information, refer to:
    - Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
    - Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.
- The use of BNG®SARS-CoV-2 IVD and data evaluation is restricted to trained laboratory personnel only.
- Good laboratory practice is essential for optimal performance of this assay. Special care must be taken avoid contamination of the components of the kit. All reagents must be closely monitored for impurities and contamination. Discard suspicious reagents according to local guidelines and regulations.

8. SPECIMENS

Only use appropriate specimens for testing, such as:

- Respiratory specimens including nasopharyngeal swabs, oropharyngeal (throat) swabs, combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes, bronchoalveolar lavage and saliva.
- Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are acceptable. Other specimens should be collected according to the procedure related to sample.

9. SPECIMENS – HANDLING AND STORAGE

- Specimens can be stored at 4^0C for up 72 hours after collection.
- If a delay in extraction is expected, store specimens at -20^0C for longer periods -70^0C or lower.
- Extracted nucleic acids should be stored at -20^0C for longer periods -70^0C or lower.

Do not use specimens if

- They were not kept at $2\text{-}4^0$C ($\leq$ 4 days) or frozen at -20^0C or for longer periods -70^0C or below.
- They are insufficiently labelled or lack documentation.
- They are not suitable for this purpose (see above for suitable sample material).
- The specimen volume is insufficient.

10. SAMPLE PREPARATION

The performance of RT-qPCR assays strongly depends on the amount and quality of sample template RNA. It is strongly recommended to qualify and validate RNA extraction procedures for recovery and purity before testing specimens.

Suitable rapid nucleic acid isolation based molecular transport medium and nucleic acid extraction systems successfully used in combination with **BNG® COVID-19 RT-qPCR Detection Kit** include: BNG® Quick RNA Isolation and Moleculer Transport Kit, BNG® Quick RNA Isolation Kit, Roche MagNA Pure Compact RNA Isolation Kit, QIAmp® Viral RNA Mini Kit, Quick-RNA Viral Kits (Zymo Research), TIANamp Virus DNA/RNA Kit and Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit.

- Only extract the number of specimens that will be tested in a single day.If the specimena4s are collected into the rapid molecular transport and isolation solution store it +4 ^{0}C for 72 hours.
- Do not freeze / thaw extracts more than once before testing as each freeze/thaw cycle will decrease the RNA quality. For optimal results, use directly and do not freeze and thaw before use.

- Extracted nucleic acids should be stored at -20^{0}C, for longer storing periods -70^{0}C or lower and (if re-testing is expected) stored in aliquots.

11. REACTION SETUP

1. Make sure that all necessary equipment and devices are suitable, calibrated, and functional before starting the experiments.
2. Decontaminate equipment and workspace and prepare everything needed for the following experiment to keep the workflow short and repeatable.
3. Switch on the qPCR detection system and program it to avoid delays after setting up the reactions.
4. Thaw all components of **BNG® COVID-19 RT-qPCR Detection Kit** on ice and mix gently but thoroughly to ensure even distribution of components. Collect liquid at the bottom of the tube with a quick spin. Make sure the sample is well mixed.
5. The mixture is calculated according to the number of samples and the mixture is prepared in a sterile microcentrifuge. It is recommended to prepare master mix for 2 additional reactions to compensate for pipetting inaccuracies.
6. Distribute 15 µL of the master mix (which includes mix and enzyme) to each well of your PCR plate.

COMPONENT	VOLUME
BNG One-Step Reaction Mix (N/Orf1ab/RdRp/S/E)and (HEC)	15 uL
Isolated Sample RNA/TPC/NTC	5 ul
	Total 20 uL

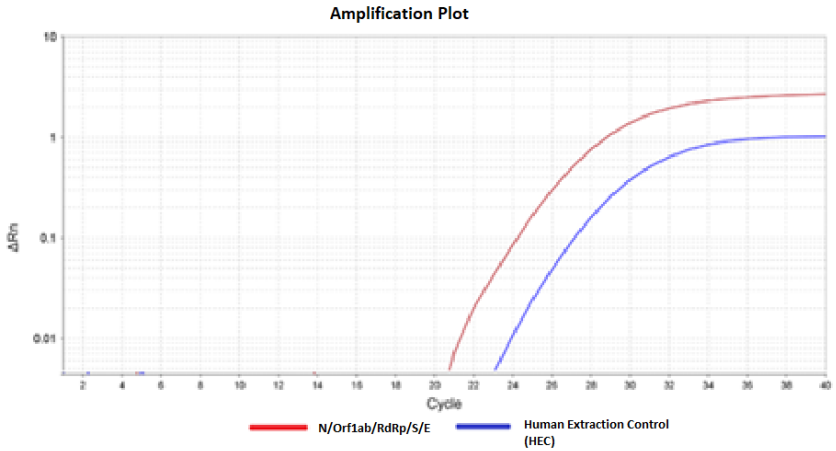
7. In the RNA samples that have been isolated, 5µL (20-100 ng) is added to the wells. Only 5uL of sample is added to each well.
8. Use the nuclease-free water provided with the kit instead of the RNA in the negative control, and the plasmid DNA provided with the kit in the positive control.
9. qPCR plate surface is sealed with a seal.
10. Transfer the reactions to the PCR device, then cycle according to the guidelines:

STEP	Cycle	Temperature	Duration
Reverse Transcription	1	55^{0}C	5 min
Initial Denaturation	1	95^{0}C	5 min
Amplification	40	95^{0}C	5 sec
		60^{0}C*	30 sec

Enable Data Collection for **FAM™** (for N/Orf1ab/RdRp/S/E virus detection), **JOE/HEX / VIC™** (GAPDH / RNaseP for human extraction control). Set the Passive Reference to ROX if necessary. (63 minutes are completed in QuantStudio 5 Real-Time PCR instrument).

11. Once the run is finished, do not open the reaction tubes to avoid contamination and discard according to local guidelines and regulations. Do not autoclave as this may contaminate laboratory equipment with amplicons.

12. ANALYSIS & TROUBLESHOOTING



** **HEX /VIC/JOE** is used for Human Extraction Control (HEC). (GAPDH/RNase P for **JOE/HEX/VIC™**) (N/Orf1ab/RdRp/S/E)for **FAM™**).**FAM** and **HEX/VIC/JOE** must be defined on the device interface for each well in the multiplex kit.

- **dH₂O controls (NTC) must not give a positive Ct for any assay.** If they do, the reaction was contaminated with sample RNA/DNA. Decontaminate equipment and workspace and repeat the reactions.
- **For a sample to be considered positive for SARS-CoV-2, any of the 2 regions (N /Orf1b/ RdRp/S/E) must give a positive Ct value.** If the human extraction control fails to amplify, the sample must still be considered positive. Ct values (< 38 cycles)
- **For a sample to be considered negative for SARS-CoV-2, none of the 2 assays (N /Orf1b/ RdRp/S/E) must give positive Ct values.** The human extraction control must give a positive Ct value (< 38 cycles) for these samples to ensure that sample material of suitable quality was present.
- **All reactions containing RNA isolate must give positive Ct values for the HEC assay. The Ct values should be < 38 cycles.** Failure to amplify the negative human extraction control indicates a flawed RNA extraction or loss of RNA isolate due to RNase contamination. The sample is not sufficient, results can not be interpreted.
- If the **patient sample** is **positive** and the **NTC** control is **negative**; contamination problem The experiment is repeated by paying attention to the points in the Warnings section.
- If the **patient sample** is **negative** and the **NTC** control is **positive**; Since the target is negative, there is no contamination problem. NTC supplied with kit contents and added to negative control sample may be contaminated. If the problem persists, the manufacturer is contacted and a new negative control is requested.
- The Threshold for **Bio-Rad** should be kept between 20-30. In **Qiagen Rotor Gene** instruments, whether the growth curves are sigmidal or not should be evaluated from the RAW DATA screen. In order to see the Ct values of sigmoidal curves in Rotor-Gene devices, DYNAMIC TUBE active SLOPE CORRECT options must be passive from the analysis screen. Outlier removel option should be 0. Threshold should be set to 0.03. Threshold values for different devices are determined during installation.
- **If FAM is positive in positive samples, HEX is negative, the sample should be considered positive.**

(FAM)	(HEX/VIC/JOE)	Interpretation
+	+	All 2 target sequences for SARS-CoV-2 and the HEC were amplified. The **sample is considered positive for SARS-CoV-2.**
+	-	Repeat the testi if the results are still the same, the patient is evaluated as **positive.**
-	+	Only the target sequence for the HEC was amplified. **The sample is considered negative for SARS-CoV-2**
-	-	PCR was inhibited, results are invalid.

13.LIMITATIONS

- For reliable results, it is essential to adhere to the guidelines given in this manual. Changes in reaction setup or cycling protocol may lead to failed experiments.
- Depending on the sample matrix, inhibitors may be present in the isolated RNA and disable reverse transcription and/or PCR amplification. If this is the case, another sample type or isolation method may be beneficial.
- Spontaneous mutations within the target sequence may result in failure to detect the target sequence.
- Results must always be interpreted in consideration of all other data gathered from a sample. Interpretation must be performed by personnel trained and experienced with this kind of experiment.
- For safety reasons, specimen collection, transport, storage, and processing procedures must be performed by trained personnel only.
- This assay must not be used on specimens directly. Appropriate rapid molecular transport and isolation kits or nucleic acid extraction methods must be conducted prior to using this assay.
- Reliable results depend strongly on proper sample collection, storage, and handling procedures.

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