For in vitro diagnostic use only.
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Cat No: BX-SY-WCOR-308-100/BX-SY-WCOR-308-250/BX-SY-WCOR-308-500/BX-SY-WCOR-308-1000

SARS-CoV-2 Triple Gene RT-qPCR Kit

Package Insert

1. Kit Content

Table 1: Kit Content

Component	Intended Use	Amount			
		100 Rxns	250 Rxns	500 Rxns	1000 Rxns
2X Prime Script Mix	One-Step RT-qPCR	1 x 500 µL	1 x 1250 µL	2 x 1250 µL	4 x 1250 µL
CVD Tri Oligo Mix	Specific amplification of the target regions in the SARS-CoV-2 and Human genome (Internal Control; IC): *ORF1ab*, *N*, *E* (FAM), and *RNase P* (HEX)	1 x 250 µL	1 x 625 µL	1 x 1250 µL	2 x 1250 µL
NTC	Negative (No Template) Control (Nuclease-Free Water)	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL
PC-CVD Tri	Positive Control (Synthetic RNA fragment mixture of the targets in the "CVD Tri Oligo Mix")	1 x 250 µL	1 x 250 µL	1 x 500 µl	2 x 500 µL

Table 2: Storage Requirements and Shelf Life

Component	Transport Conditions	Storage Conditions	Shelf Life
2X Prime Script Mix	+2 - +8 °C	-20 °C	12 months
CVD Tri Oligo Mix	+2 - +8 °C	-20 °C	
NTC	+2 - +8 °C	+2 - +8 °C/-20 °C	
PC-CVD Tri	+2 - +8 °C	before opening -20 °C, after first thaw +2 - +8 °C	

! Each reagent stored at storage temperature, can be used until the expiration date indicated on the tube following the first opening. The expiration date of the kit is determined by the expiration date of the reagents.

2. Materials Required but Not Provided

Table 3: Components Required but not Included with the Test

Components Required but not Included with the Test	
1. Real-Time instrument with FAM and HEX channels, Ramp rate ≥3 °C/sec	**7.** Reaction tubes and their caps/seals compatible with the qPCR instruments and the reaction volume
2. Adjustable micropipettes and compatible pipet tips (nuclease-free)	**Extra components recommended to use:**
3. Centrifuge	**8.** Biosafety cabinet for PCR setup
4. Vortex	**9.** Cold tube rack (for microcentrifuge tubes and PCR tubes/strips)
5. Nuclease-free water/viral transport medium/serum physiologic	**10.** PPE (Personal Protective Equipment)
6. 1.5- or 2-mL microcentrifuge tubes (nuclease-free)	

3. Intended Use and Test Principle

BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit is an one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the presumptive qualitative detection of RNA from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, oral/saliva swabs, bronchoalveolar lavage, nasopharyngeal aspirates, saliva, gargle, and sputum samples from individuals suspected of COVID-19 by their healthcare provider or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection.

Detection with the kit is achieved via rapid nucleic acid extraction from respiratory tract samples followed by multiplex real-time RT-PCR targeting the SARS-CoV-2 specific *Open Reading Frame 1ab* ***(ORF1ab)***, *Nucleocapsid* ***(N)***, and *Envelope* ***(E)*** genes and human ***RNase P*** mRNA in real-time PCR instruments that are equipped with FAM and HEX detection channels. Fluorescent signals from the ***ORF1ab***, ***N*** and ***E*** genes create a cumulative effect in the FAM channel, increasing the sensitivity of SARS-CoV-2 detection. **The kit allows to achieve RT-qPCR result in less than 30 minutes (Run time may vary depending on the instrument).**

The oligonucleotide set targeting human ***RNase P*** mRNA functions as a control of the sampling, nucleic acid extraction, reverse transcription, and qPCR since the oligonucleotide set targets the exon-exon junction. The kit also contains negative and positive control templates for testing the contamination and the qPCR reactive stability, respectively.

BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit is a moderate complexity test. It is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.

4. Analytical Specifications

BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit is validated with ***Zybio EXM3000 Nucleic Acid Isolation System*** *(Robot Catalog No: ZBI-EXM3000)* and ***vNAT® Extraction Consumables*** (***vNAT® Transfer Tube*** *(Catalog No: BS-NA-513-100 and BS-NA-513m)* and ***vNAT® Viral Nucleic Acid Buffer*** *(Catalog No: BS-NA-510))* for nucleic acids extracted from nasopharyngeal swabs, oropharyngeal swabs, oral/saliva swabs, bronchoalveolar lavage, nasopharyngeal aspirates, saliva, gargle, and sputum samples.

The RT-qPCR is carried out in 10 µL reaction volume using the ***CFX96 Touch™/CFX96™ Dx (Bio-Rad), CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad), QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™),*** and ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)*** Real-Time PCR systems equipped with the FAM and HEX detection channel. The data produced by the instruments can manually be evaluated and reported using their software or can automatically be evaluated and reported using the online ***FastFinder*** software: https://www.ugentec.com/fastfinder.

For the analysis performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems. **The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments compared to the white tubes.** Besides, device-specific reaction tubes should be used on ***BMS*** and ***Applied Biosystems™*** instruments (the specified analytical performance of the kit can only be achieved using the validated tubes).

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Limit of Detection (LoD) of the ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit*** is 500 copies/mL for nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples in the ***vNAT® Transfer Tube***, 1000 copies/mL for nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples in the VTM extracted using the ***vNAT® Viral Nucleic Acid Buffer*** and 500 copies/mL for bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples in the VTM extracted using the ***Zybio EXM3000 Nucleic Acid Isolation System***.

The exclusivity of the kit was tested on 43 different viral and bacterial strains and a pool of nasal washes from 20 different healthy people. The kit does not cross-react with other respiratory pathogens and human respiratory microbial flora. Sensitivity and specificity of the kit relative to an FDA authorized RT-qPCR assay was tested on 1030 (222 positive and 808 negative) clinical samples and the results are given in Table 4. The relative sensitivity and specificity of the kit were determined as 100.00% and 100.00%, respectively.

Table 4: Clinical Performance

All respiratory specimens		FDA authorized RT-qPCR assay		
		Positive	Negative	Total
BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit	Positive	222	0	222
	Negative	0	808	808
	Total	222	808	1030
Positive Predictive Value		(222/222) x 100 = 100.00%		
Negative Predictive Value		(808/808) x 100 = 100.00%		

5. Collection, Storage and Shipment of Clinical Specimens

Nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples should be collected using Dacron or polyester flocked swabs by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Swabs should be placed immediately into the ***vNAT® Transfer Tube*** containing 2 mL of the ***vNAT® reagent*** or into a sterile transport tube containing 2-3 mL of viral transport medium (VTM) (Preparation of viral transport medium, Centers for Disease Control and Prevention, SOP#: DSR-052-01). Bronchoalveolar lavage and nasopharyngeal aspirate sample types should be transferred into sterile tube containing 2-3 mL of VTM. Other sample types (saliva, gargle, and sputum) should be transferred into preservative-free sterile tubes.

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Shipping regulations for UN 3373 Biological Substance, Category B must be followed when sending potential 2019-nCoV specimens. Store the specimens in the VTM or preservative-free sterile containers at +2 - +8°C and ship to the laboratory on ice pack. The specimens in the ***vNAT® Transfer Tube*** can be stored and transferred to the laboratory at room temperature within 24 hours. For transfers longer than 24 hours, ship the specimens to the laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to the laboratory on dry ice. It is important that specimens are not exposed to continuous freeze-thaw exposure.

After collection, specimens in the VTM or preservative-free sterile container can be stored at +2 - +8°C for up to 72 hours and specimens in the ***vNAT® Transfer Tube*** can be stored at +2 - +8°C for up to 3 months. If a delay in the RT-qPCR test is expected, store specimens at -70°C or lower in accordance with the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19.

6. Warnings

1. Specimen processing should be performed in accordance with national biological safety recommendations.
2. Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
3. All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate. Sampling should be carried out by personnel with sufficient knowledge and experience.
4. The kit should be stored away from nucleic acid sources and PCR amplicons.
5. Except for fluid transfers, nucleic acid and positive control tubes should always be kept closed.
6. To prevent contamination of the reaction mixture by previously amplified target sequences, maintain separate work areas, dedicated equipment.
7. Different sets of laboratory coats should be worn pre- and post-PCR.
8. The micropipettes used for pipetting PCR mixes and template nucleic acids should be separate. Filtered and nuclease-free tips should be used.
9. For collection of nasopharyngeal/oropharyngeal swabs, Dacron or polyester flocked swabs are preferred. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some viruses and inhibit PCR.
10. It is recommended to use swabs with breakable shaft to prevent contamination during sampling.
11. The components in the kit should not be mixed with different lot numbers or chemicals of the same name but from different manufacturers.
12. Master stock reagents should be kept on the cold block during the PCR setup.
13. Kit components should be mixed by gently shaking before use.
14. Maintenance/calibration interval should be determined for all instruments and equipment used with the kit.
15. To avoid false positives due to amplified material, the PCR completed reaction tubes should be disposed of before opening in the laboratory.
16. The wipeable surfaces of the rooms, benches, and devices should be cleaned regularly with freshly diluted 10% bleach solution (0.5% NaClO).
17. Dispose of waste in a designated matter in accordance with local, regional, and federal regulations.

7. RT-qPCR Application Protocol

Before starting the assay, please consider the following:

1. The kit was validated only for the template nucleic acid volume that is 25% of the total qPCR volume.
2. The kit cannot be used with real-time PCR instruments without the periodic maintenance records.
3. For ***QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*** instruments **"Passive Reference Dye"** should be **"None"** selected.
4. **It is recommended to use validated qPCR plate/strip with the kit!** The specified analytical performance of the kit can only be achieved using the validated tubes.
5. **For testing the contamination, setup two different negative control reactions with and without addition of NTC.**

Program the qPCR device as follows and add the reagents to the qPCR tubes, close the tubes, place them into the qPCR instrument and start the run (Table 5).

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Table 5: Reaction Set-up and RT-qPCR Program Details

Reaction Setup		RT-qPCR Program							
		CFX96 Touch™/CFX96™ Dx (Bio-Rad), CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad), and Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)				*QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*			
Reagent	**Volume/Rxn**	**Step**	**Cycle No.**	**Temperature**	**Duration**	**Step**	**Cycle No.**	**Temperature**	**Duration**
2X Prime Script Mix	5 µL	Reverse Transcription	1	52 °C	3 min	Reverse Transcription	1	52 °C	5 min
		Hold	1	95 °C	10 sec				
CVD Tri Oligo Mix	2.5 µL	Denaturation	5	95 °C	1 sec	Hold	1	95 °C	10 sec
		Annealing/Extension		60 °C	12 sec				
Template Nucleic Acid	2.5 µL	Denaturation	35	85 °C	1 sec	Denaturation	40	95 °C	1 sec
		Annealing/Extension		60 °C	1 sec	Annealing/Extension		60 °C	12 sec
TOTAL REACTION VOLUME	10 µL	**Detection (Reading)**	**Instrument**	***ORF1ab+N+E* (FAM)/*RNase P* (HEX)**		**Detection (Reading)**	**Instrument**	***ORF1ab+N+E* (FAM)/*RNase P* (HEX)**	
			Bio-Rad	FAM (492/517), HEX (530/556)			Applied Biosystems	FAM (470/520), VIC (520/558)	
			BMS	Green (465/510), Yellow (540/570)					

8. Interpretation of the Assay Results

- The threshold level should be set to 200 RFU for ***CFX96 Touch™/CFX96™ Dx (Bio-Rad)*** and ***CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments to calculate Ct values. All other default analysis options in the related software should not be changed for ***CFX96 Touch™/CFX96™ Dx (Bio-Rad)*** and ***CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)*** instruments. For ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)***, **"Non-Assay Green/Parameters/Dynamic"** and **"Auto-Threshold"** options should be selected to calculate Ct values. **"Auto-Threshold"** options should be selected to calculate Ct values for ***QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*** instruments.
- When the run is finalized by the qPCR instrument, the produced data file is uploaded to the online ***FastFinder*** software to interpret the results as described below.
- Shape of the amplification curves obtained in the **FAM**/**HEX** channels should be examined for all reaction wells returning with Ct values. Ct values should be used in the further interpretation steps if their amplification curve shapes are sigmoidal. **Non-sigmoidal curves should be recorded as negative.** The result is recorded as positive if Ct ≤ 33.
- For samples with a suspected sigmoidal curve pattern under the threshold in the **FAM** channel, Ct-**HEX** (IC) should be examined. If the Ct-**HEX** ≤30, the sample is reported as negative. If the Ct-**HEX** >30, the test should be repeated after freezing and thawing the sample. If the problem continues after the freezing and thawing, a new sample is requested.
- **Because of 40 cycles of PCR reaction applied for *QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)* Real-Time PCR systems; It should be interpreted by adding 5 cycles to the Ct values given in Table 6 (Expected Performance of the Kit Controls) and Table 7 (Interpretation of Patient Samples).**

Table 6: Expected Performance of the Kit Controls

Control Type	Control Name	Purpose	Expected Results and Ct Values	
			***RNase P* (HEX)**	***ORF1ab+N+E* (FAM)**
Negative Control	NTC	Contamination control during RT-qPCR	**Negative** (No Ct)	**Negative** (No Ct)
No template addition	NRC	Reactive contamination control	**Negative** (No Ct)	**Negative** (No Ct)
Positive Control	PC	Reagent integrity	**Positive** (Ct ≤ 33)	**Positive** (Ct ≤ 33)
Internal/Extraction Control [1]	IC	To monitor the integrity of nucleic acid extraction and RT-qPCR from each human respiratory tract specimen	**Positive** (Ct ≤ 33)	If IC Ct>33.0, and if target Ct≤33.0, conclude as IC is valid

[1] **When evaluating the patient samples results, if the *ORF1ab+N+E* target is positive with a Ct value ≤ 33 and with a negative *RNase P* result, it is considered that there is no inhibition, extraction or sampling problem and the run is valid.**

If any control does not perform as described above, the run is considered invalid, and the test is repeated.

1. **Invalid PC (Ct>33 in any channel):** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC (Ct≤33 in any channel):** Repeat the analysis by paying attention to the "Warnings" section.
3. **Invalid NRC (Ct≤33 in any channel):** Contact the manufacturer, renew the reagents, and repeat the reaction.
4. **Invalid IC (Ct>33 in HEX channel):** Repeat the analysis by increasing the reaction volume to 50 µL by keeping the ratios of the reaction components in Table 5. If the problem continues, then conclude as an invalid PCR template.

Assessment of clinical specimen test results must 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.

Table 7: Interpretation of Patient Samples

***ORF1ab+N+E*/FAM (Positive for Ct ≤ 33)**	***RNase P*/HEX (Positive for Ct ≤ 33)**	**Results Interpretation**	**Action**
Positive (+)	Positive (+)	Results are VALID, SARS-CoV-2 RNA is detected	Report as **POSITIVE**
Positive (+)	Negative (-)	Results are VALID, SARS-CoV-2 RNA is detected	Report as **POSITIVE**
Negative (-)	Positive (+)	Results are VALID, SARS-CoV-2 RNA is not detected	Report as **NEGATIVE**
Negative (-)	Negative (-)	Results are INVALID (sampling/extraction/inhibition problem)	Re-extract the specimen and perform testing again. If the result is still invalid, a new specimen should be obtained. If additional clinical sampleis unavailable, report as **INVALID**

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WARNING: On the web page linked with the QR code, examples of the sigmoidal amplification curves are given. The results obtained with this kit should **NOT** be interpreted without examining these samples.

WARNING: For detailed information please refer to Instruction for Use (IFU).

9. Limitations

- ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit*** is a moderate complexity test. It is intended for use by laboratory personnel trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Performance of the ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit*** has only been established in nasopharyngeal swab, oropharyngeal swab, oral/saliva swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, gargle, and sputum samples. Combined nasopharyngeal/oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates and nasal washes are also considered acceptable specimen types, but performance has not been established.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false negative results since they may contain substances that inactivate some viruses and inhibit PCR. Dacron or polyester flocked swabs are recommended for collection of swab samples. Performance of the ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit*** has only been evaluated using Dacron or polyester flocked swabs.
- Mutations within the target regions of the ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit*** could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Inhibitors or other types of interference may produce a false negative result. Mucin at 50% (w/v), blood at 50% (v/v), nasal spray (Nasonex) at 10% (v/v), nasal corticosteroids and gels at 10% (w/v), throat lozenges at 10% (w/v), anti-viral at 1% (v/v), antibiotics at 0.1% (w/v) may interfere with the ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit***. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- Detection of SARS-CoV-2 RNA may be affected by patient factors (e.g., presence of symptoms), and/or stage of infection.
- Based on the *in-silico* analysis, other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the ***BioeXsen SARS-CoV-2 Triple Gene RT-qPCR Kit.*** Other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 are not known to be currently circulating in the human population, therefore are highly unlikely to be present in-patient specimens.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.

10. Explanation of Symbol

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
CE	European Economic Area	LOT	Batch code		Do not use if package is damaged and consult instructions for use
IVD	For *In Vitro* Diagnostic Use	REF	Catalog Number		Keep away from water/moisture
	Manufacturer	NON STERILE	Non-Sterile		Temperature limit (Storage temperature)
	Use-by Date (Expiration Date) YYYY-MM		Consult Instructions for Use		Keep it upright
CONTROL -	Negative Control		Caution	Σ	Total number of IVD tests that can be performed with the IVD medical device
CONTROL +	Positive Control		Keep away from light		
CONTROL	Control		Protect from heat and radioactive sources		

11. Manufacturer and Technical Support

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