For in vitro diagnostic use only.
For professional use only.

Release and Revision Date: 03 2021/07
BEGZ3121v3

100 Rxns | T00099-T00099
250 Rxns | T00100-T00100
500 Rxns | T00101-T00101
1000 Rxns | T00102-T00102

BioeXsen Health Technology

CE IVD

BioeXsen SARS-CoV-2 Double Gene RT PCR Package Insert

1. Reagents and Materials Provided

Table 1 Kit Content

Component	Amount				Intended Use
	100 Rxns	250 Rxns	500 Rxns	1000 Rxns	
2X Prime Script Mix	1 x 500 µL	1 x 1250 µL	2 x 1250 µL	4 x 1250 µL	One-Step RT-qPCR
CVD Di Oligo Mix	1 x 250 µL	1 x 625 µL	1 x 1250 µL	2 x 1250 µL	Specific amplification of the target region in the SARS-CoV-2 and human genomes: *ORF1ab* and *N* (**FAM**), *RNase P* (**HEX**)
NTC	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	No Template (Negative) Control
PC-CVD Di	1 x 250 µL	1 x 250 µL	1 x 500 µl	2 x 500 µL	Positive Control

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 Di Oligo Mix	+2 - +8 °C	-20 °C	
NTC	+2 - +8 °C	-20 °C	
PC-CVD Di	+2 - +8 °C	before opening -20 °C, after opening +2 - +8 °C	

Each reagent stored at storage temperature, can be used until the expiration date indicated on the tube. 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. qPCR Cycler **2.** Adjustable micropipettes and compatible tips **3.** Centrifuge **4.** Vortex machine **5.** 1.5 or 2 mL microcentrifuge tubes, nuclease-free **6.** Swabs for nasopharyngeal, oropharyngeal, and nasal swab samples	**7.** Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume. **Extra components recommended to use:** **8.** UV Cabinet for PCR Setup **9.** Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips) **10.** PPE (Personal Protective Equipment)

3. Intended Use and Test Principle

BioeXsen SARS-CoV-2 Double Gene RT PCR is a one-step reverse transcription and real-time PCR (rRT-PCR) test intended for the presumptive qualitative detection of RNA from 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, saliva and gargle 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 *ORF1ab* and *nucleocapsid (N)* 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* and *N* genes create a cumulative effect in the FAM channel, increasing the sensitivity of SARS-CoV-2 detection.
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 (Dekker, Rob J., et al. Overhauling a faulty control in the CDC-recommended SARS-CoV-2 RT-PCR test. bioRxiv, 2020). The kit also contains negative and positive control templates for testing the contamination and the qPCR reactive stability, respectively.

4. Performance Evaluation

LoD of the ***BioeXsen SARS-CoV-2 Double Gene RT PCR*** is 500 copies/mL for nasopharyngeal dacron swab, nasopharyngeal polyester flocked swab, oropharyngeal dacron swab, and oropharyngeal polyester flocked swab samples in *BioeXsen* **vNAT®** *Transfer Tube* and 1000 copies/mL for nasopharyngeal dacron swab, nasopharyngeal polyester flocked swab, oropharyngeal dacron swab, oropharyngeal polyester flocked swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva, and gargle samples in VTM.

The exclusivity was tested wet with Coronavirus 229E/OC43/NL63/HKU1, MERS, SARS-CoV strain Frankfurt 1, Influenza A H1/H3, Influenza B, Parainfluenza 1/2/3/4, Metapneumovirus, Rhinovirus, Respiratory syncytial virus (RSV) A/B, Bocavirus (BoV), Enterovirus, Adenovirus, *Legionella pneumophila, Chlamydia pneumoniae, Mycobacterium tuberculosis, Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma pneumoniae, Streptococcus pyogenes, Bordetella pertussis, Pneumocystis jirovecii, Candida albicans, Legionella bozemanii, Legionella micdadei, Corynebacterium diphtheriae, Bacillus anthracis, Moraxella catarrhalis, Neisseria meningitidis, Pseudomonas aeruginosa, Staphylococcus epidermidis, Coxiella burneti, Staphylococcus aureus, Streptococcus salivarius, Leptospira interrogans, Chlamydia psittaci* and a pooled nasal wash from 20 different people (healthy donors) combined with the negative clinical swab sample pool was also used in the exclusivity study to test diverse microbial flora in the human respiratory tract.

Tabel 4 and 5: Clinical Performance

Nasopharyngeal (NP) swab samples from individuals without symptoms or other reasons to suspect COVID-19		FDA authorized Xpert® Xpress SARS-CoV-2 (Cepheid)		
		Positive	*Negative*	*Total*
BioeXsen SARS-CoV-2 Double Gene RT PCR	*Positive*	27	0	27
	Negative	0	673	673
	Total	27	673	700
Positive Percent Agreement		(27/27) x 100 = 100%		
Negative Percent Agreement		(673/673) x 100 = 100%		

All respiratory specimens including alternative specimens (Saliva and Gargle samples) from patients suspected of COVID-19		FDA authorized Xpert® Xpress SARS-CoV-2 (Cepheid)		
		Positive	*Negative*	*Total*
BioeXsen SARS-CoV-2 Double Gene RT PCR	*Positive*	*274*	*0*	*274*
	Negative	*1*	*240*	*241*
	Total	*275*	*240*	*515*
Positive Percent Agreement		(274/275) x 100 = 99.64%		
Negative Percent Agreement		(240/240) x 100 = 100%		

5. Collection, Storage and Shipment of Clinical Specimens

Swab samples should 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. The swab samples should be placed immediately into the BioeXsen ***v*NAT®** Transfer Tube 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). Other sample types should be transferred into sterile containers containing VTM. Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Specimens can be stored at +2 - +8 °C for up to 3 days (72 h) after collection. If a delay in extraction 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. Clinical specimens in BioeXsen ***v*NAT®** Transfer Tube can be stored at +2 - +8 °C for 3 months. For long term storage extracted nucleic acid should be stored at -70 °C or lower. It is important to avoid repeated freezing and thawing of specimens.

6. Warnings

1. Store the kit away from nucleic acid sources and qPCR amplicons.
2. Do not mix the kit components with different lot numbers or chemicals of the same name but from different manufacturers.
3. Keep the master stock reagents on the cold block during the PCR setup.
4. If it is possible, setup PCR on the cold block.
5. Mix the kit components gently before use.
6. Use separate micropipettes for pipetting qPCR mixes and template nucleic acids.
7. Always keep the template nucleic acid and positive control tubes closed, except for the fluid transfers.
8. Regularly clean the wipeable surfaces of the rooms, benches, and devices where the test is performed with 10% NaOCl.
9. Disposed of the qPCR completed reaction tubes before opening in the laboratory.

In the RT PCR protocol, fluorescence readings are not made in the first 5 cycles. Therefore, 5 cycles have to be added to Ct values detected by the software when reporting results.

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. It is recommended to use validated qPCR plate/strip with the kit!
4. **For testing the contamination, setup negative control reaction with 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 6).

Table 6 Reaction set-up and RT-qPCR program details.

Reaction Setup		Reaction volume: 10 µL			
Reagent	Volume/ Rxns	Cycle No.	Step	Temperature	Duration
2X Prime Script Mix	5 µL	1	Reverse Transcription	52 °C	3 min
		1	Hold	95 °C	10 sec
CVD Di Oligo Mix	2.5 µL	5	Denature	95 °C	1 sec
			Anneal/Extend	60 °C	12 sec
		35	Denature	85 °C	1 sec
			Anneal/Extend	60 °C	1 sec
Template Nucleic Acid	2.5 µL	Detection (Reading)			

Instrument	*ORF1ab/N (FAM)*	*RNase P (HEX)*
Bio-Rad	FAM (450-490/ 515-530)	HEX (515-535/ 560-580)
Roche	FAM (470/514)	HEX (533/572)
Qiagen	Green (470/510)	Yellow (530/555)
BMS	Green (465/510)	Yellow (540/570)
HiMedia	FAM (470/525)	HEX (523/564)

8. Interpretation of the Assay Results

- The threshold levels should be set to 0.05 RFU for LightCycler® 96 and 200 RFU for CFX96 Touch™ instruments to calculate Ct values. All other default analysis options in the related software should not be changed for LightCycler® 96 and CFX96 Touch™ instrument. "Non-Assay Green/ Parameters/ Fixed Length" and "Auto-Threshold" options should be selected to calculate Ct values for Mic qPCR Cycler. "Dynamic Tube" should be active, "Slope Correct" should be passive, "Outlier Removal" option should be "0" and "Threshold Level" should be set to 0.02 RFU to calculate Ct values for Rotor-Gene® 5 Plex.
- 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. The analysis is repeated with the same nucleic acid extract if Ct > 33, if the result is Ct > 33 again, a new sample from the patient is taken.
- In the RT PCR protocol, fluorescence readings are not made in the first 5 cycles. Therefore, 5 cycles can be added to Ct values detected by the software when reporting results.

Table 7. Expected performance of the kit controls.

Control Type	Control Name	Purpose	Expected Results and Ct Values	
			ORF1ab/N (FAM)	*RNase P* (HEX)
No Template (Negative) Control	NTC	Contamination control during RT-PCR	**Negative** (No Ct)	**Negative** (No Ct)
Positive Control	PC	Reagent integrity	**Positive** (Ct ≤ 33)	**Positive** (Ct ≤ 33)
Internal/ Extraction Control	IC	To monitor the integrity of nucleic acid extraction and RT-PCR from each human respiratory tract specimen	Not applicable	**Positive** (Ct ≤ 33)

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

1. **Invalid PC:** It is recommended to contact the manufacturer, renew the reagents, and repeat the reaction.
2. **Invalid NTC:** Repeat the analysis by paying attention to the "**Warnings**" section.
3. **Invalid IC:** Repeat the analysis. If residual specimen is available, test is performed again. If the re-tested sample does not give a positive result in the HEX channel, a new specimen should be collected from the patient.

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.

The assay results are interpreted as given in Table 8. The results can be interpreted as "SARS-CoV-2 Positive" as long as there is no sigmoidal amplification curve in the negative control. The results can be interpreted as "SARS-CoV-2 Negative" as long as there is a sigmoidal amplification curve with a Ct ≤ 33 in the internal and positive controls.

Table 8. Interpretation of Patient Samples

ORF1ab/N 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 sample is unavailable, report as **INVALID**

9. Limitations

- ***BioeXsen SARS-CoV-2 Double Gene RT PCR*** is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR 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 Double Gene RT PCR*** has only been established in nasopharyngeal swab, oropharyngeal (throat) swab, bronchoalveolar lavage, nasopharyngeal aspirate, saliva and gargle 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. Flocked (polyester) or dacron swabs are recommended for collection of nasopharyngeal/ oropharyngeal swab samples. Performance of the ***BioeXsen SARS-CoV-2 Double Gene RT PCR*** has only been evaluated using dacron and polyester flocked swabs.
- Mutations within the target regions of the ***BioeXsen SARS-CoV-2 Double Gene RT PCR*** 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 Double Gene RT PCR***. 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 Double Gene RT PCR***. 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.

10. Manufacturer and Technical Support

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Symbol	Meaning	Symbol	Meaning
CE	European Conformity		Temperature limit (Store temperature)
IVD	For *In vitro* Diagnostic Use		Keep away from light
REF	Catalog Number		Keep away from water/moisture
LOT	Lot Number (Batch Code)	NON STERILE	Non-Sterile
	Manufacturer		Keep it upright
	Use-by Date (Expiration Date)		Consult Instructions for Use