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

Release and Revision Date: 03 2021/07
BEVPZ3121v3

100 Rxns | T00315-T00315
250 Rxns | T00314-T00314
500 Rxns | T00319-T00319
1000 Rxns | T00316-T00316

BioeXsen Health Technology

CE IVD

BioeXsen SARS-CoV-2 Variant Plus 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 1250 µL	2 x 1250 µL	4 x 1250 µL	One-Step RT-qPCR
Variant Oligo Mix	--	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), *S_E484K* (ROX) mutation, and *N_D3L* (CY5) mutation
NTC	--	1 x 250 µl	1 x 500 µl	1 x 1000 µL	No Template (Negative) Control
PC- Variant	--	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 (up to 5 days)	-20 °C	12 months
Variant Oligo Mix	+2 - +8 °C (up to 5 days)	-20 °C	
NTC	+2 - +8 °C (up to 5 days)	+2-8 °C / -20 °C	
PC- Variant	+2 - +8 °C (up to 5 days)	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

The ***BioeXsen SARS-CoV-2 Variant Plus*** kit is a one-step reverse transcription and real-time PCR (RT-qPCR) test intended for the qualitative detection of RNA from the SARS-CoV-2 and the detection of the SARS-CoV-2 N_D3L mutation (SARS-CoV-2 lineage B.1.1.7) and the S_E484K mutation (SARS-CoV-2 lineages B.1.351, P.1, B.1.525, B.1.526 etc.) in nasopharyngeal swab, oropharyngeal swab, and nasal swab 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. The ***BioeXsen SARS-CoV-2 Variant Plus*** kit allows going from sample to SARS-CoV-2 detection result in less than 30 minutes while differentiating the emerging variants without the need for the DNA sequencing.

In a single multiplex reaction, the ***BioeXsen SARS-CoV-2 Variant Plus*** kit targets the *ORF1ab* and *N* gene regions found in all SARS-CoV-2 for the routine screening as well as the *N_D3L* mutation for the B.1.1.7 detection and the *S_E484K* mutation for the detection of the variants with a high possibility of escaping the antibody-mediated immunity. The human *RNase P* oligo set in the kit targets exome-exome junction in the mRNA and does not target the human genome. Hence it is used for controlling the sampling, integrity of RNA, nucleic acid extraction, and inhibition of both reverse transcription and qPCR. 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 Variant Plus for the NPDS, NPFS, OPDS, OPFS, NDS and NFS samples is 500 SARS-CoV-2/mL for the *ORF1ab/N* target, 1000 SARS-CoV-2/mL for the *N_D3L* target, and 10000 SARS-CoV-2/mL for the *S_E484K* target.

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.

Table 4 and 5: Clinical Performance

All respiratory specimens from from individuals without symptoms or other reasons to suspect COVID-19		FDA authorized RT-PCR *(BioeXsen SARS-CoV-2 RT PCR)*		
		Positive	*Negative*	*Total*
BioeXsen SARS-CoV-2 Variant Plus	*Positive*	23	0	23
	Negative	0	277	277
	Total	23	277	300
Positive Percent Agreement		(23/23) x 100 = 100%		
Negative Percent Agreement		(277/277) x 100 = 100%		

All the respiratory specimens from patients suspected of COVID-19		FDA authorized RT-PCR comparator test		
		Positive	*Negative*	*Total*
BioeXsen SARS-CoV-2 Variant Plus	*Positive*	*118*	*0*	*118*
	Negative	*0*	*282*	*282*
	Total	*118*	*282*	*400*
Positive Percent Agreement		(118/118) x 100 = 100%		
Negative Percent Agreement		(282/282) 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
Variant 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) *CFX96 Touch™*		*ORF1ab/N (FAM)*	*RNase P (HEX)*	*S_E484K (ROX)*	*N_D3L (CY5)*

8. Interpretation of the Assay Results

The threshold level should be set to 200 RFU and all other default analysis settings in the Bio-Rad CFX Maestro™ software should not be changed. Shape of the amplification curves obtained in the FAM/HEX/ROX/CY5 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 have to 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)	*S_E484K* (ROX)	*N_D3L* (CY5)
No Template (Negative) Control	NTC	Contamination control during RT-PCR	**Negative** (No Ct)	**Negative** (No Ct)	**Negative** (No Ct)	**Negative** (No Ct)
Positive Control	PC	Reagent integrity	**Positive** (Ct ≤ 33)	**Positive** (Ct ≤ 33)	**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)	*Not applicable	*Not applicable

* 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.

If all the controls are valid, proceed to the interpretation of the Ct results. Check Ct of the targets in FAM, ROX and CY5 channels:

- If **Ct-FAM is ≤33, conclude as positive**, **otherwise conclude as negative**.
- If **Ct-HEX is ≤33, conclude as positive**, **otherwise conclude as negative**.
- If **Ct-ROX is ≤33**, **conclude as positive**, **otherwise conclude as negative**.
- If **Ct-CY5 is ≤33**, calculate the **difference between Ct-CY5 and Ct-FAM**. If the **difference is <6**, **conclude as positive for the CY5**, **otherwise conclude as negative for the CY5**.

After the interpretation of the Ct results, interpret the lineage results as described in Table 8.

Table 8 Interpretation of Patient Samples

Case	*ORF1ab/N* (FAM)	*RNase P* (HEX)	*S_E484K* (ROX)	*N_D3L* (CY5)	Result
1	-	+	-	-	**1) SARS-CoV-2 is negative.**
2	+	+	-	-	**1) SARS-CoV-2 is positive.** **2)** Alpha variant (B.1.1.7) and the S_E484K containing SARS-CoV-2 variants (Beta (B.1.351), Gamma (P.1), Eta (B.1.525), and Iota (B.1.526) etc.) are negative.
3	+	+	+	-	**1) SARS-CoV-2 is positive.** **2)** Alpha variant (B.1.1.7) is negative. **3)** The detected SARS-CoV-2 contains the S_E484K mutation (Beta (B.1.351), Gamma (P.1), Eta (B.1.525), and Iota (B.1.526) etc.).
4	+	+	-	+	**1) SARS-CoV-2 is positive** **2)** The detected SARS-CoV-2 variant contains the N_D3L mutation. Lineage of the variant is B.1.1.7 with probability more than 99% (Alpha). **3)** The detected SARS-CoV-2 variant does not contain the S_E484K mutation.
5	+	+	+	+	**1) SARS-CoV-2 is positive.** **2)** The detected SARS-CoV-2 variant contains the N_D3L mutation. Lineage of the variant is B.1.1.7 with probability more than 99% (Alpha). **3)** The detected SARS-CoV-2 variant contains the S_E484K mutation.
6	-	-	-	-	Perform the test again. If the result is still invalid, a new specimen should be obtained. If additional clinical sample is unavailable, report as **INVALID**

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.

9. Limitations

- ***BioeXsen SARS-CoV-2 Variant Plus*** 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 Variant Plus*** has only been established in nasopharyngeal, oropharyngeal and nasal swab samples. Combined nasopharyngeal/ oropharyngeal swabs 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 Variant Plus*** has only been evaluated using dacron and polyester flocked swabs.
- Mutations within the target regions of the ***BioeXsen SARS-CoV-2 Variant Plus*** 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 50% (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 Variant Plus***. 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.

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