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

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Cat No: BX-SY-WCOR-403-250/BX-SY-WCOR-403-500/BX-SY-WCOR-403-1000

SARS-CoV-2 Variant Plus V2

Package Insert

1. Reagents and Materials Provided

Table 1: Kit Content

Component	Intended Use	Amount		
		250 Rxns	500 Rxns	1000 Rxns
2X Prime Script Mix	One-Step RT-qPCR	1 x 1250 µL	2 x 1250 µL	4 x 1250 µL
Emerging Oligo Mix	Specific amplification of the target region in the SARS-CoV-2 and Human genomes: *SARS-CoV-2, ORF1ab+N (FAM), Human RNase-P mRNA (IC) (HEX), Spike (S) E484K mutation (ROX), Nucleocapsid (N) D3L mutation (CY5), Spike (S) L452R mutation (CY5.5)*	1 x 625 µL	1 x 1250 µL	2 x 1250 µL
NTC	Negative (No Template) Control	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL
PC-Emerging	Positive Control	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
Emerging Oligo Mix	+2 - +8 °C	-20 °C	
NTC	+2 - +8 °C	+2 - +8 °C/-20 °C	
PC-Emerging	+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 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, HEX, ROX, CY5, and CY5.5 channels 2. Adjustable micropipettes and compatible tips (nuclease-free) 3. Centrifuge 4. Vortex 5. Swabs for nasopharyngeal, oropharyngeal, and oral/saliva samples and sterile containers containing VTM for the other sample types 6. Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume	7. 1.5- or 2-mL microcentrifuge tubes (nuclease-free) 8. Nuclease-free water, ***vNAT®*** *Transfer Tube* [1] or ***vNAT®*** *Viral Nucleic Acid Buffer* [2] and Viral Transport Medium (VTM) **Extra components recommended to use:** 9. UV Cabinet for PCR Setup 10. Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips) 11. PPE (Personal Protective Equipment)

[1] The ***vNAT®*** *Transfer Tube* (Cat No: BS-NA-513 and BS-NA-513m) is used for the preservation and transportation of the samples collected with dry swab. No nucleic acid extraction is required.
[2] The ***vNAT®*** *Viral Nucleic Acid Buffer* (Cat No: BS-NA-510) is used for the nucleic acid extraction from the samples collected in VTM.

3. Intended Use and Test Principle

SARS-CoV-2 variants that have mutations changing the transmission rate, disease severity and immune system response are termed Variant of Interest (VOI) by the World Health Organization (WHO). Among the VOIs those that pose a global threat are moved to the Variant of Concern (VOC) list, thus countries are warned about the danger that is approaching. The most up-to-date list can be found at https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.

The "***BioeXsen SARS-CoV-2 Variant Plus V2***" kit tests and distinguishes SARS-CoV-2, the Alpha variant, the Delta variant and the E484K mutants in a **single multiplex RT-qPCR reaction in less 30 minutes**. The kit is applied to the nasopharyngeal swabs, oropharyngeal swabs, oral/saliva swabs, bronchoalveolar lavage, nasopharyngeal aspirates, saliva, gargle, and sputum samples taken by healthcare providers from the COVID-19 suspected individuals.

The "***BioeXsen SARS-CoV-2 Variant Plus V2"*** kit, besides targeting the ***Orf1ab*** and ***N*** gene regions which are common in all SARS-CoV-2 variants, targets the ***N D3L*** mutation for the detection of the **Alpha** Variant, targets the ***S L452R*** mutation for the **Delta** variant detection, and targets the ***S E484K*** mutants that are mainly **Gamma** and **Mu** variants. There have been ***231576 SARS-CoV-2*** genome entries to the global GISAID database since the beginning of the 2022. 12187 of the genomes belongs to Alpha, Beta, Gamma and Delta variants. The remaining amount is **219411 genomes, 217681 genomes of which are the *Omicron variant*. In other words, if the detected variant is not one of the Alpha, Beta, Gamma and Delta variants, it is an Omicron variant with 99.2% probability.**

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. The Alpha and Delta variants have high transmission rate, whilst the E484K mutants and the Delta variant imply the re-infection risk. In addition, testing these variants via RT-qPCR will decrease the load of genotyping via DNA sequencing.

4. Analytical Specifications

The ***BioeXsen SARS-CoV-2 Variant Plus V2*** is validated with the ***Zybio EXM3000 Nucleic Acid Isolation System (Robot Catalog No: ZBI-EXM3000)*** *and* ***vNAT®*** *Extraction Consumables (****vNAT®*** *Transfer Tube Cat No: BS-NA-513* and *BS-NA-513m;* ***vNAT®*** *Viral Nucleic Acid Buffer Cat No: BS-NA-510).* Clinical swab samples collected from individuals suspected of respiratory tract viruses (e.g., SARS-CoV-2, Influenza, HMPV, RSV etc.) are transferred 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). Nucleic acids are extracted using the ***vNAT®*** *Viral Nucleic Acid Buffer* from the

samples collected in VTM. 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) and QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*** Real-Time PCR systems equipped with the FAM, HEX, ROX, CY5, and CY5.5 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 analyzes performed on *Bio-Rad Real-Time PCR systems*, the kit has been validated with white reaction tubes specific to these systems *(Cat No: 1845098, TLS0851, TCS0803/Bio-Rad Laboratories Inc.* and *Cat No: BS1001, BS2001/EndeXs Health Solutions LLC)*. The clear reaction tubes result in 5-10 times lower fluorescence signal in the *Bio-Rad* instruments when compared to the white tubes. Besides, device-specific reaction tubes should be used on Applied Biosystems instruments (the specified analytical performance of the kit can only be achieved using the validated tubes).

The SARS-CoV-2 genomes used for the oligonucleotide design reflect all the major lineages and the important variants emerged recently. The designed oligonucleotide sequences match 100% with all their targets in the GISAID database. **Limit of detection (LOD) of the "*BioeXsen SARS-CoV-2 Variant Plus V2*" kit for SARS-CoV-2 is 500 copies/mL and 10000 copies/mL for N_D3L, S_E484K, and S_L452R.** The kit's results were negative for all the 43 bacterial and viral strains and a pooled nasal wash from the healthy donors. The in-silico tests also revealed that the oligonucleotide sets of the assay did not cross-react any nucleotide sequence in the database. The inclusivity was tested with the archived positive clinical nasopharyngeal samples from 107 different SARS-CoV-2 lineages. The tested samples were sequenced via Next Generation Sequencing (NGS). The RT-qPCR test results were in 100% agreement with the sequencing results.

The "***BioeXsen SARS-CoV-2 Variant Plus V2***" was applied to 500 clinical specimens (129 positive + 371 Negative) concurrently with an FDA EUA approved RT-qPCR kit. The relative sensitivity and specificity of the kit is 100%. Next Generation Sequencing (NGS) was also applied to 129 clinical samples determined to be positive, and the relative specificity of the kit to NGS results was determined as 100%.

5. Collection, Storage and Shipment of Clinical Specimens

Nasopharyngeal swab, oropharyngeal swab, and oral/saliva swab samples should be collected using dacron or polyester 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 VTM. Other sample types (saliva, gargle, and sputum) should be transferred into preservative-free sterile tube.

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 containers can be stored at +2 - +8ºC for up to 72 hours and specimens in the ***vNAT®*** *Transfer Tube* can be stored at +2 - +8ºC 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 4).

Table 4: 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)				*QuantStudio™ 5, 0.1 mL/QuantStudio™ 5 Dx, 0.1 mL (Applied Biosystems™)*			
Reagent	**Volume/Rxns**	**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				
Emerging Oligo Mix	2.5 µL	Denature	5	95 °C	1 sec	Hold	1	95 °C	10 sec
		Anneal/Extend		60 °C	12 sec				
Template Nucleic Acid	2.5 µL	Denature	35	85 °C	1 sec	Denature	40	95 °C	1 sec
		Anneal/Extend		60 °C	1 sec	Anneal/Extend		60 °C	12 sec
TOTAL REACTION VOLUME	10 µL	**Detection (Reading)**	**FAM / HEX / ROX / CY5/ CY5.5 Read**			**Detection (Reading)**	**FAM / VIC/ ROX / CY5/ CY5.5 Read**		

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.*** "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/ROX/CY5/CY5.5 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/ROX/CY5/CY5.5 channels, 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™); It should be interpreted by adding 5 cycles to the Ct values given in Table 5 (Expected performance of the kit controls) and all patient results.**

Table 5: Expected performance of the kit controls

Control Type	Name	Control purpose	Expected Result	
			IC (HEX)	SARS-CoV-2 (FAM), Variants (ROX/CY5/CY5.5)
NTC addition	NTC	Contamination control	No Ct = Valid	
No template addition	NRC	Reactive contamination control	No Ct = Valid	
PC addition	PC	Positive reactive control	Ct≤33.0 = Valid	
Human mRNA	IC	Control of the sampling, RNA integrity, nucleic acid extraction, inhibition of both reverse transcription and qPCR	Ct-HEX ≤ 32.0 = Valid	Ct-HEX > 32.0 Ct-FAM/ROX/CY5/CY5.5 ≤ 32.0 = Valid

If any control does not perform as described in Table 4, the run is considered invalid, and the test is repeated.

1. Invalid PC: Contact the manufacturer, renew the reagents, and repeat the reaction.
2. Invalid NRC: Contact the manufacturer, renew the reagents, and repeat the reaction.
3. Invalid NTC: Repeat the analysis by paying attention to the "**Warnings**" section.
4. Invalid IC: Repeat the analysis by increasing the reaction volume to 50 µL by keeping the ratios of the reaction components in Table 4. If the problem continues, then conclude as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the Ct results. Check Ct of the targets in FAM, ROX, CY5 and CY5.5 channels:

- If **Ct-FAM 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**, **conclude as positive**, **otherwise conclude as negative**.
- If **Ct-CY5.5 is ≤33**, **conclude as positive**, **otherwise conclude as negative**.

After the interpretation of the Ct results, interpret the lineage results as described in Table 6.

Table 6. Interpretation of Patient Samples

Case	FAM	ROX	CY5	CY5.5	Result
Case 1	-	-	-	-	**1)** SARS-CoV-2 is negative.
Case 2	+	-	-	-	**1)** SARS-CoV-2 is positive. **2)** Alpha and Delta variants and the E484K containing variants are negative.
Case 3	+	+	-	-	**1)** SARS-CoV-2 is positive; **2)** One of the E484K containing variants (Check Table 2) is positive. **3)** Alpha and Delta variants are negative.
Case 4	+	+	+	-	**1)** SARS-CoV-2 is positive. **2)** Alpha variant containing the E484K mutation is positive. **3)** Delta variant is negative.
Case 5	+	+	-	+	**1)** SARS-CoV-2 is positive; **2)** Delta variant containing the E484K mutation is positive. **3)** Alpha variant is negative.
Case 6	+	-	+	-	**1)** SARS-CoV-2 is positive **2)** Alpha variant is positive. **3)** The E484K containing variants and Delta variant are negative.

Case 7	+	-	-	+	**1)** SARS-CoV-2 is positive. **2)** Delta variant is positive. **3)** Alpha variant and the E484K containing variants are negative.
Case 8	+	-	+	+	**1)** SARS-CoV-2 is positive. **2)** Delta variant containing the N D3L mutation is positive. **3)** The E484K containing variants are negative.

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 V2*** 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 V2*** 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. Flocked (polyester) or dacron swabs are recommended for collection of swab samples. Performance of the ***BioeXsen SARS-CoV-2 Variant Plus V2*** has only been evaluated using dacron and polyester flocked swabs.
- Mutations within the target regions of the ***BioeXsen SARS-CoV-2 Variant Plus V2*** 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 Variant Plus V2.*** 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 Variant Plus V2.*** 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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