Cat no: BS-SY-WCOR-402-250/BS-SY-WCOR-402-500/BS-SY-WCOR-402-1000

SARS-CoV-2 Variant Plus

Package Insert

Bio-Speedy®

CE IVD

1. Kit content

Shelf Life: 12 months; refer to the expiration date on the box. 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.

Table 1a. Kit content

Storage Temperature: -20°C, Transport Temperature: +2-8°C					
Content/Intended Use	**Content**	**Quantity (10 µL Reactions)**			**Consumption / Reaction**
		250 Reactions	**500 Reactions**	**1000 Reactions**	
SARS-CoV-2, ORF1ab+N (**FAM**) Human RNase-P mRNA (IC) (**HEX**) B.1.1.7 / B.1.351 / P.1 (**ROX**) B.1.1.7 (**CY5**)	Variant Oligo Mix	1 x 625 µL	1 x 1250 µL	2 x 1250 µL	2.5 µL
DNA polymerase, dNTP mix, reverse transcriptase enzyme, ribonuclease inhibitor and reaction buffer mix	2X Prime Script Mix	1 x 1250 µL	2 x 1250 µL	4 x 1250 µL	5 µL

Table 1b. Kit content-Controls

Storage Temperature: +2-8°C/-20°C; Transport Temperature +2-8°C/-20°C If the components are frozen store at -20°C. Store at 2-8 °C after the first thawing.					
Negative Control Template **Test it in each run for contamination control**	NTC	1 x 1000 µL	1 x 1000 µL	1 x 1000 µL	2.5 µL
Positive Control Template: Synthetic SARS-CoV-2 and VOC-202012/01 genom fragment **Test it in each run for reactive stability control**	PC-Variant	1 x 250 µL	1 x 500 µL	2 x 500 µL	2.5 µL

Instruments and equipment supplied by the user	
1. Real-Time PCR Instrument: 4 channel, Ramp rate ≥3 °C/sec. **2.** 1-10 µL, 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips (DNase and RNase free) **3.** Quick Spin Centrifuge: min. 3000 rpm **4.** Vortex **5.** Nuclease-free water/Viral Transport Medium/Serum physiologic	**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.** Disposable powder-free nitrile gloves

2. Intended Use and Test Principle

The "***Bio-Speedy® 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 SARS-CoV-2 and the differentiation of the B.1.1.7, B.1.351 and P.1 SARS-CoV-2 lineages in nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, nasopharyngeal aspirates, saliva and bronchoalveolar lavage samples from individuals suspected of COVID-19 by their healthcare provider.

In a single multiplex reaction, the "***Bio-Speedy® 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 specific genomes regions only found in B.1.1.7, B.1.351 and P.1. The human RNase-P oligo set 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.

Because the B.1.1.7, B.1.351 and P.1 SARS-CoV-2 lineages are important for epidemiology, the timely and representative estimation of their prevalence by testing a much higher portion of the suspected cases in much lower time compared to the sequencing-based technologies is necessary. The RT-qPCR-based "***Bio-Speedy® SARS-CoV-2 Variant Plus***" kit allows going from **sample to SARS-CoV-2 detection result in 40 minutes** while differentiating the variants. In addition, quantity of the qPCR instruments has been increasing tremendously while its application cost has been decreasing all around the world.

B.1.1.7 is correlated with a significant increase in the rate of COVID-19 infection in the United Kingdom, and could be up to 70% more transmissible than previous variants due to the unusually large number of mutations. Spike N501Y is of particular importance among the other mutations of B.1.1.7, because it is in the C-terminal of receptor binding domain (RBD) of the S protein. In addition to the N501Y mutation, the B.1.351 and P.1 lineages carry the Spike E484K and K417(T/N) mutations in the C-terminal of RBD. 28 of the 59,056 available B.1.1.7 genomes also contain the E484K mutation (Retrieved from GISAID on 09.02.2021). Escape from neutralizing antibodies by the spike protein E484K variants was previously reported using a recombinant chimeric SARS-CoV-2 reporter virus. Molecular dynamic simulations have pointed out the possibility that the combination of E484K, K417N and N501Y mutations induces conformational change greater than N501Y mutant alone. Genomic data showed that B.1.351 rapidly displaced other lineages circulating in South Africa, and preliminary studies suggest the variant is associated with a higher viral load, which may suggest potential for increased transmissibility. It was also reported that one of the available vaccine provides limited protection against COVID-19 infection from B.1.351. Hence, entry of the new variants' carriers should be prevented at the country borders, filiation studies should be accelerated for the identified cases and more specific quarantine conditions should be created. This is possible only by **testing the highest portion of the suspected cases in the shortest time** by using RT-qPCR based kits like the "***Bio-Speedy® SARS-CoV-2 Variant Plus***".

3. Analytical Specifications

The "***Bio-Speedy® SARS-CoV-2 Variant Plus***" kit is validated with the "***vNAT™ Viral Nucleic Acid Buffer (BS-NA-510)***" and "***vNAT™ Transfer Tube (BS-NA-513-100)***". The kit is validated for 10 µL qPCR volume using *Bio-Rad CFX96 Touch™, Bio Molecular Systems Magnetic Induction Cycler (MIC), Qiagen Rotor-Gene® 5 Plex* and *HiMedia Insta Q96® Plus* Real-Time PCR systems.

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 "***Bio-Speedy® SARS-CoV-2 Variant Plus***" kit is 1000 copies/mL for all the sample types and targets. The kit's results were negative for all the 42 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 kit was applied to 309 clinical samples containing SARS-CoV-2 from 39 different lineages that were determined via the NGS (GISAID accession IDs between 428712-428723, 429861-429873, 437304-437335, 811136-811143, 812761-812781, 812873-812921, 814062-814091, 894246-894276, 903347, 906049, 935048-935102, 939627, 940633-940682, 940709-940713). The RT-qPCR results were in 100% agreement with the NGS results that 116 samples were B.1.1.7 positive, 2 samples were B.1.351 positive, and 191 samples were negative for B.1.1.7, B.1.351 and P.1. The specificity of the "***Bio-Speedy® SARS-CoV-2 Variant Plus***" kit relative to the NGS is 100%.

4. Collection, Storage and Shipment of Clinical Specimens

Swab samples should be collected using Dacron or Polyester swabs. Other specimen types should be transferred in sterile containers. In the transport phase, Viral Transport Medium (VTM) (Preperation of viral transport medium, Center for Disease Control and Prevention, SOP#: DSR-052-01) or *Bio-Speedy® vNAT™ Viral Transfer Tubes (Cat No:BS-NA-513-100)* should be used. Samples should be stored and transported at 2-8 °C until they arrive at the laboratory. Swab samples should be transferred within 5 days, other sample types should be transferred within 2 days. If a delay in shipment is expected, samples should be frozen at -70°C and shipped with dry ice. It is important that samples should not be exposed to the repeated freeze-thaw.

5. 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% NaClO.
9. Disposed of the qPCR completed reaction tubes before opening in the laboratory.

6. 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. Do not use qPCR plates/strips that is not recommended by the manufacturer of the qPCR instrument.
4. **For testing the contamination, setup two different negative control reactions with 1) no template addition, and 2) addition of NTC.**

Program the qPCR device as follows and add the reagents to the qPCR tubes in the order specified below, close the tubes, place them into the qPCR device and start the run (Table 2).

Table 2. Reaction set-up and qPCR program details

Reaction setup		**qPCR Program**		
Component	**Reaction**	**Cycle Number**	**Temperature**	**Duration**
2X Prime Script Mix	5 µL	1	52°C	5 min
Variant Oligo Mix	2.5 µL	1	95°C	10 sec
		40	95°C	1 sec
Template Nucleic Acid	2.5 µL		60°C	5 sec
TOTAL REACTION VOLUME	10 µL		FAM/HEX/ROX/CY5 Read*	

***Alternative dyes in different instruments for reading: FAM: GREEN, HEX: YELLOW / VIC, ROX: ORANGE / TEXAS RED, CY5: RED**

7. Interpretation of The Assay Results

The recommended threshold level to calculate the number of threshold cycles (Cq) is 200 RFU for *Insta Q96® Plus* and *CFX96 Touch™* instruments. "**Dynamic Tube**" should be active, "**Slope Correct**" should be passive, "**Outlier Removal**" should be **"0"**, and the threshold level should be set to 0.02 to calculate the Cq values in *Rotor-Gene® Q.* For *BMS MIC* qPCR, "**Non-Assay Green/Parameters/Dynamic**" options should be selected, auto-threshold setting should be active.

The shape of the amplification curves should be examined. If a Cq value is assigned to a sample by the instruments' software and the curve is sigmoidal, the Cq value can be used in the final evaluation. **Non-sigmoidal curves should be recorded as negative.** If a Cq value is assigned to a sample, but the curve is not sigmoidal, **the result should be recorded as 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.

Table 3. Expected performance of the kit controls

Control Type	**Name**	**Control purpose**	**Expected Result**	
			IC (HEX)	**SARS-CoV-2 (FAM), Variants (ROX/CY5)**
NTC addition	NTC	Contamination control	No Cq = Valid	
No template addition	NRC	Reactive contamination control	No Cq = Valid	
PC addition	PC	Positive reactive control	Cq<38.0 = Valid	
Human mRNA	IC	Control of the sampling, RNA integrity, nucleic acid extraction, inhibition of both reverse transcription and qPCR	Cq≤34.0 = Valid	If IC Cq ≥34.0, and if SARS-CoV-2 or Variant Cq≤34.0, conclude as IC is valid

If any control does not perform as described above, 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 2. If the problem continues, then conclude as an invalid PCR template.

If all the controls are valid, proceed to the interpretation of the results. **Check Cq of the targets in FAM, ROX and CY5 channels. If the Cq is ≤38, conclude as positive** for the target, otherwise conclude as negative. Interpret the results as described in Table 4.

For samples with a suspected sigmoidal curve pattern under the threshold in FAM, ROX or CY5 channels, Cq value of the IC should be examined. If the IC Cq≤34, the sample is reported as negative. If the Cq≥34, 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.

Table 4. Interpretation of Patient Samples

Case	FAM	ROX	CY5	Result
Case 1	-	-	-	1) SARS-CoV-2 negative
Case 2	+	-	-	1) SARS-CoV-2 positive; 2) B.1.1.7, B.1.351 and P.1 negative
Case 3	+	+	+	1) SARS-CoV-2 B.1.1.7 positive; 2) B.1.351 and P.1 negative
Case 4	+	+	-	1) SARS-CoV-2 B.1.351 or P.1 positive; 2) B.1.1.7 negative

8. Limitations

- Performance of the *Bio-Speedy® SARS-CoV-2 Variant Plus* has only been established in nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, nasopharyngeal aspirates, saliva and bronchoalveolar lavage samples.
- Mutations within the target regions of the *Kit* could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Inhibitors or other types of interference may produce a false negative result. 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 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.

9. Manufacturer and Technical Support

Bioeksen R&D Technologies Limited Company

Address: ITU ARI Teknokent 3, 4/B-105, 34467. Sariyer, Istanbul, TURKEY.
Phone: +90 (212) 285 10 17
Fax: +90 (212) 285 10 18
Web: www.bioeksen.com.tr
e-mail: info@bioeksen.com.tr, support@bioeksen.com.tr