SARS-CoV-2 British Variant Real-TM

Handbook

Real Time PCR kit for detection of main SARS-CoV-2 British Variant mutations present in lineage 1.1.7

REF **V435-96FRT/B**

96

NAME

SARS-CoV-2 British Variant Real-TM

INTRODUCTION

Viruses continuously change through mutation, and new variants of a virus are expected to emerge over time. Multiple variants of the SARS-Cov-2 virus that causes COVID-19 have been documented globally during this pandemic.

The virus that causes COVID-19 is a new type of coronavirus, a large family of viruses. Coronaviruses owe their name to the crown-like spikes on their surfaces. Scientists monitor virus changes, especially changes to the spikes on the surface of the virus. These studies, including genetic analyses, are helping scientists understand how changes to the virus might affect how it spreads and what happens to people who are infected with it.

Many variants of the virus that causes COVID-19 are circulating globally: the United Kingdom (UK) identified a variant called B.1.1.7 carrying different mutations in the fall of 2020. This variant can spread more easily and quickly than other variants. In January 2021, experts in the UK reported that this variant may be associated with an increased risk of death compared to other variant viruses, even if more studies are necessary to confirm this finding. It has since been detected in many countries around the world.

INTENDED USE

SARS-CoV-2 British Variant Real-TM is Real-Time PCR test for the qualitative detection of RNA in clinical samples of SARS-CoV-2 coronavirus lineage B.1.1.7 (British variant) main mutations, in the ORF1ab and Spike (S) genes.

PRINCIPLE OF ASSAY

SARS-CoV-2 British Variant Real-TM test is based on three major processes: isolation of *virus* RNA from specimens, reverse transcription of the RNA, Real Time amplification of the cDNA.

The kit is intended for screening of positive samples for SARS-CoV-2 and for the presence of SARS-CoV-2 coronavirus lineage B.1.1.7 (VOC 202012/01) by detecting the following gene mutations: ORF1ab: 11288-11296 deletion (SGF 3675-3677), Spike (S): 21765- 21770 deletion (HV 69-70 DEL) in RNA preparations isolated from human biomaterial (nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal and nasopharyngeal aspirates, sputum).

SARS-CoV-2 British Variant Real-TM PCR kit is a multiplex Real Time PCR kit with three simultaneous targets: SARS-CoV-2 wild type (ROX channel), ORF1ab: 11288-11296 deletion (SGF 3675-3677) (Cy5/Red channel) and Spike (S): 21765- 21770 deletion (HV 69-70 DEL) (Cy5.5 channel).

Detection channel	ROX/Orange	Cy5/Red	Cy5.5
Result	**SARS-Cov-2 WT ("wild type")** S gene	**ORF1ab**: 11288-11296 deletion (SGF 3675-3677) - ***mutation***	**Spike (S)**: 21765-21770 deletion (HV 69-70 DEL) - ***mutation***

MATERIALS PROVIDED

Format S (strip format)

- **Strips-COVID19 British Variant**, 8x12 strip tubes (15 µl in each tube), including optical strip caps;
- **RT-PCR Buffer**, 2 x 0,81 ml;
- **Enzymes Taq/RT**, 0,055 ml;
- **Pos cDNA C+**, 0,13 ml;

Contains reagents for 96 tests.

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA extraction kit
- Real Time qPCR Thermalcycler instrument
- Workstation
- Pipettes with aerosol barrier
- Tubes and tubes racks

STORAGE INSTRUCTIONS

All reagents of **SARS-CoV-2 British Variant Real-TM kit** must be stored at 2-8°C except for **Enzymes Taq/RT vial** which has to be stored at -20°C. The kits can be shipped at 2-8°C for 3-4 days but should be stored at -20°C (or 2-8°C) immediately on receipt.

STABILITY

SARS-CoV-2 British Variant Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification. Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

WARNINGS AND PRECAUTIONS

The user should always pay attention to the following:

- Clinical specimens from Sars-Cov-2 cases should be considered as biological substances and must be handled in a BSL-2 laboratory
- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

SARS-CoV-2 British Variant Real-TM can analyze RNA extracted from:

- *Nasopharyngeal / nasal swabs*: swab area and place in "Eppendorf" tube with 0,5 ml of saline water or PBS sterile (Sacace Transport medium is recommended). Agitate vigorously. Repeat the swab and agitate in the same tube. Use 100 µl of solution for RNA extraction.
- *Tracheal aspirate, bronchial lavage, nasal wash:* centrifuge at 10000 g/min for 10-15 min. If the pellet is not visible add 10 ml of liquid and repeat centrifugation. Remove and discard the supernatant. Resuspend the pellet in 100 µl of Saline water.

Specimens can be stored at +2-8°C for no longer than 48 hours, or frozen at -20°C to -80°C for longer periods. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

RNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the "SAMPLE COLLECTION, STORAGE AND TRANSPORT" paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

⇨ **M-Sorb-S** (Sacace, REF K502/100/A);

⇨ **DNA/RNA Prep NA** (Sacace, REF K-2-9/2);

⇨ **QIAmp™ DSP Viral RNA Mini Kit** (Qiagen®, , REF 61904);

⇨ **SaMag Viral Nucleic Acids Extraction kit** (Sacace, REF SM003)

Please carry out the RNA extraction according to the manufacturer's instructions.

Extracted RNA should be processed immediately or frozen at -20°C for up to 1 week (max 1 defrosting).

ONE STEP REVERSE TRANSCRIPTION AND PCR AMPLIFICATION **(40 µl reaction volume)**

1. Prepare required quantity of PCR tubes or PCR strips according to the number of samples to be analyzed, 1 tube for negative control of extraction (NCE), 1 tube for negative control of amplification (C-) and 1 tube for positive control of amplification (C+) (for example, to test 5 samples, mark 8 tubes)
2. Prepare in a new tube **Reaction Mix** with **15*N µl** of **RT-PCR Buffer** and **0,5*N µl** of **Enzymes Taq/RT**, for N tubes to be tested. Vortex the tube thoroughly. Then spin briefly for 3-5 sec.
   Mixture of RT-PCR-mix and Enzyme Taq/RT must be prepared immediately prior to use and can be stored at the temperatures from 2 °C to 8 °C for 1 hour.
3. Add **15 µl** of prepared **Reaction Mix** into each PCR tube, without touching the wax layer.
4. Add **10 µl** of extracted **RNA** sample to the appropriate tube with Reaction Mix, without touching the wax layer, close the tubes with provided caps, spin 2-3 seconds, then transfer them to the qPCR instrument.
5. Prepare for each panel 3 controls:
   - add **10 µl** of **cDNA C+** to the tube labeled C+ *(positive control of amplification)*;
   - add **10 µl** of **Negative Control buffer*** to the tube labeled C- *(negative control of amplification)*;

**not provided, use DNA/RNA free water.*

Amplification

1. Create a temperature profile on your instrument as follows:

	Plate-type qPCR Instruments[1]		
Step	**Temperature, °C**	**Time**	**Cycles**
1	35	20 min	1
2	94	5 min	1
3	94	10 sec	5
	64	25 sec	
4	94	10 sec	45
	64	25 sec **Fluorescence detection** **	

** **NOTE FOR CFX-96** and other plate type instruments: it is recommended to use at least 3 strips in each run placing them in the left, center and right columns of the thermal block to better uniform the thermolid pressure in case of not filling the complete plate.*

[1] For example, SaCycler-96™ (Sacace), CFX-96™(BioRad); Mx3005P™ (Agilent),*

*** Fluorescence detection on channels ROX, Cy5, Cy5.5*

SARS-CoV-2 Wild Type is detected on the **Rox (Orange) channel, SARS-CoV-2 ORF1ab deletion** is detected on the **Cy5 (Red) channel** and **SARS Spike 69-70 deletion** is detected on the **Cy5.5 channel.**

Detection channel	ROX/Orange	Cy5/Red	Cy5.5
Result	**SARS-CoV-2 WT ("wild type")** S gene	**ORF1ab**: 11288-11296 deletion (SGF 3675-3677) - ***mutation***	**Spike (S)**: 21765-21770 deletion (HV 69-70 DEL) - ***mutation***

INSTRUMENT SETTINGS

Plate-type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold in the log-linear phase of amplification, approximately 10-20% of the fluorescence level of the positive control in the last amplification cycle.

RESULTS ANALYSIS

When using SaCycler-96 software, results are analysed automatically in the software RealTime PCR. For manual analysis of results refer to Table 1, Table 2 and text below.

Table 1. Results interpretation

Detection channel			**Interpretation of the result**
Rox	Cy5	Cy5.5	
Analyzed samples			
Ct≤35*	Ct not defined, or Δ Ct (Cy5-Rox) ≥5	Ct not defined, or Δ Ct (Cy5.5 -Rox) ≥5	**SARS-CoV-2 "wild type" RNA detected. No detectable mutations of SARS-CoV-2 lineage B.1.1.7**
Ct not defined, or Δ Ct (Rox-Cy5.5) ≥5	**Ct≤35***	**Ct≤35**	**Mutations of SARS-CoV-2 lineage B.1.1.7 detected ****
Ct not defined	Ct not defined	Ct not defined	SARS-CoV-2 RNA not detected ***
Positive control sample			
Ct defined	**Ct defined**	**Ct defined**	The result is positive The session is valid
Negative control			
Ct not defined	Ct not defined	Ct not defined	The result is negative The session is valid

** If the lowest Ct is more than 35, the sample cannot be interpreted correctly. Re-isolation of RNA and RT-PCR or re-assay of biomaterial should be performed.*

*** The simultaneous detection of two detectable mutations in the SARS-CoV-2 coronavirus RNA preparation is the basis for further research by automatic cDNA sequencing according to Sanger method in order to confirm the presence of all other mutations characteristic of SARS-CoV-2 lineage B.1.1.7.*

**** For samples, biomaterials for which the status of the presence of SARS-CoV-2 RNA is unknown, it is recommended to confirm the test result with a set of reagents for detecting only SARS-CoV-2 coronavirus RNA, in accordance with the established procedures.*

Table 2. Other possible results

Detection channel			Interpretation of the result
Rox	Cy5	Cy5.5	
Analyzed samples			
Ct not defined, or Δ Ct (Rox-Cy5.5) ≥5	Ct not defined, or Δ Ct (Cy5-Rox) ≥5	**Ct≤35***	**Mutation SARS-CoV-2 gene S: 21765-21770 deletion (HV 69-70 DEL) detected ****
Ct≤35*	**Ct≤35, or** Δ Ct (Cy5 - Rox)|≤2	Ct not defined, or Δ Ct (Cy5.5 -Rox) ≥5	**Mutation SARS-CoV-2 mutation in the ORF1ab gene: 11288-11296 deletion (SGF 3675-3677) detected ****

** If the lowest Ct is more than 35, the sample cannot be interpreted correctly. Re-isolation of RNA and RT-PCR or re-assay of biomaterial should be performed.*

*** results should be further investigated using sequencing to identify possible new lineages of SARS-Cov-2 variants*

PERFORMANCE CHARACTERISTICS

Analytical sensitivity

Limit of detection: 10 copies of nucleic acid per amplification tube.

The detection limit was established by analyzing serial dilutions of two batches of a laboratory control sample (LCS).

The detection limit of 10 copies of nucleic acid per amplification tube corresponds to the following values of the RNA concentration:

Biomaterial	Analytical sensitivity
Swab from the nasopharynx, oropharynx in 500 µl of transport medium;	500 copies/ml
Bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate	500 copies/ml

Analytical specificity

The absence of nonspecific positive amplification results was shown in the presence of *influenza A virus, influenza B virus, parainfluenza viruses types 1-4, Rhinovirus, Adenovirus, Metapneumovirus, coronaviruses HKU 1, NL63, OC43, 229E, DNA Chlamydia pneumoniae, Mycoplasma pneumoniae, Haemophilus influenza, Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Moraxella catarrhalis*, as well as human DNA, at a concentration of up to 10^8 copies / ml of the sample.

In the course of research and development, 45 positive samples of SARS-CoV-2 RNA isolated from clinical samples (swabs from the nasopharynx and oropharynx) were studied during the diagnostic and treatment process.

Diagnostic sensitivity and specificity

Number of studies (n) - 301.
Diagnostic sensitivity (95% CI) - 100% (95.49-100%);
Diagnostic specificity (95% CI) - 100% (98.34-100%).

Biomaterial type	**Diagnostic sensitivity (95% CI)**	**Diagnostic specificity (95% CI)**
Nasopharyngeal and oropharyngeal swabs, n = 226	100 % (92,89-100,00)	100 % (97,93-100,00)
Bronchoalveolar lavage, n = 19	100 % (69,15-100,00)	100 % (66,37-100,00)
Endotracheal and nasopharyngeal aspirate, n = 29	100 % (69,15-100,00)	100 % (82,35-100,00)
Sputum, n = 27	100 % (69,15-100,00)	100 % (80,49-100,00)

Interfering substances

The presence of PCR inhibitors in a sample of biological material may cause dubious (uncertain) results. A sign of PCR inhibition may be a simultaneous lack of amplification of the internal control and of a specific amplification product.

According to the results of risk analysis and R&D, the following substances are classified as PCR inhibitors, which may be present in extracted RNA: hemoglobin, which is present in the RNA sample as a result of incomplete removal of the blood-containing biomaterial, as well as isopropyl alcohol and methyl acetate present in the RNA sample as a result of incomplete removal of washing solutions during RNA extraction process.

The maximum concentrations of interfering substances at which there was no effect on the amplification of the laboratory control sample and of the internal control sample are:

- hemoglobin - 0.35 mg / ml cDNA sample
- isopropyl alcohol - 100 µl / ml cDNA sample
- methyl acetate - 100 µl / ml cDNA sample

Impurities contained in the biomaterial sample, such as mucus, blood, elements of tissue breakdown and inflammation, local drugs, including those contained in nasal sprays, etc. are removed during the isolation of RNA using kits for sample preparation. To reduce the number of PCR inhibitors, it is necessary to follow the rules for sampling the biological material. If there is a doubt of the presence of a large number of PCR inhibitors in the sample, it is recommended to choose appropriate methods for isolating nucleic acids that allow maximum removal of PCR inhibitors; express methods for isolating nucleic acids are not recommended.

TROUBLESHOOTING

1. Weak or absent signal in all channels: retesting of the sample is required.
    - The PCR was inhibited.
        ⇒ Make sure that you use a recommended RNA extraction method and follow the manufacturer's instructions.
    - The reagents storage conditions didn't comply with the instructions.
        ⇒ Check the storage conditions
    - The PCR conditions didn't comply with the instructions.
        ⇒ Check the PCR conditions select the fluorescence channel reported in the protocol.
    - The incorrect treatment of clinical material, incorrect RNA extraction, which resulted in the loss of RNA, or by the presence of PCR inhibitors.
        ⇒ Repeat RNA extraction process, take attention during the sample preparation.
2. Any signal with signal with Negative Control of Amplification (C-).
    - Contamination during PCR procedure. All samples results are invalid.
        ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        ⇒ Use only filter tips during the extraction procedure. Change tips among tubes.
        ⇒ Repeat the RNA extraction with the new set of reagents.
        ⇒ Pipette the Positive controls at the end.
        ⇒ Repeat the PCR preparation with the new set of reagents.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
RUO	For Research Use Only	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
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* MX3005P® is a registered trademark of Agilent Technologies
* QIAmp™ is a registered trademark of Qiagen

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