gb **SARS-CoV-2**
Variant SA/BR, UK

Cat. no.: 3239-100
3239-500

CE
IVD

generi biotech

PURPOSE OF USE

gb SARS-CoV-2 Variant SA/BR, UK kit enables multiplex detection of SARS-CoV-2 virus RNA (Wuhan coronavirus 2019) in RdRp gene and simultaneously the detection of mutations A570D and E484K in S gene. The A570D mutation occurs exclusively in lineage B.1.1.7 (a.k.a 20I/501Y.V1) referred to as the British variant. The E484K is an escape mutation that causes reduced binding of neutralizing antibodies against spike protein and occurs most frequently in lineages B.1.351 (a.k.a 501Y.V2) referred to as the South African variant and lineage P.1 (a.k.a 501Y.V3) referred to as the Brazilian variant. False negativity is checked by exogenous positive control with artificial sequence.

PRINCIPLE OF THE TEST

The test is based on a **one-step RT-qPCR** methodology. The kit contains all the necessary components to perform the test.

INSTRUCTIONS FOR USE

1) Let the reagents thaw completely, mix them thoroughly and spin down briefly prior each use.

Performing RNA isolation

2) Incorporate **10 µl** of **EPC Template RNA** (tube with a yellow cap) into the each isolation reaction.
3) Using **Deionized Water** as a sample with **10 µl** of **EPC Template RNA** in a separate negative isolation control is recommended.
4) Perform the RNA isolation according to your standard laboratory isolation protocol.

Performing RT-qPCR

5) Pipette **10 µl** (× number of samples) of **Master Mix OneStep Multi** (tube with a blue cap) and **5 µl** (× number of samples) of **Assay CoV-2 SA/BR, UK** (tube with a green cap) into the new tube (not included), mix well, spin down, and mark the date of preparation. In this way a **complete assay** is prepared. Handle the mixture according to the instructions in the chapter Storage and manipulation conditions.

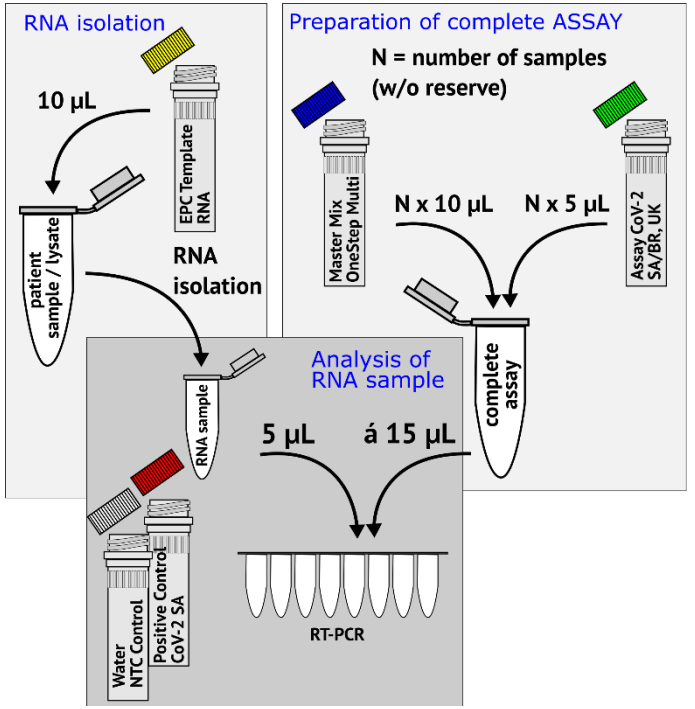
   Alternative step: In the case where the **EPC Template RNA** has not been added into the isolation, it can be used as an internal positive control in PCR by adding of **0.25 µl** (× number of samples) into the complete assay.
6) Dispense the prepared complete assay by **15 µl** into micro tubes or plate wells.
7) Add **5 µl** of template to the assay and spin briefly.
   - For each run, an analysis of **Positive Control CoV-2 SA** and **Deionized Water** as the NTC control is required for proper evaluation.
   - Use RNA prepared according to the steps described above as a template.
   - Analyse RNA as soon as possible after isolation.
8) Perform sample analysis immediately after reaction mixture preparation.

Amplification protocol and data collection

- Set the PCR cycler to the following temperature profile:

Reverse transcription	42 °C	10 min	
Initial denaturation	95 °C	3 min	
Denaturation	95 °C	10 sec	45 cycles
Annealing + Elongation (+ fluorescence acquisition)	60 °C	30 sec	

- The total volume of a PCR reaction is **20 µl**; please consider this fact when setting up the cycler.
- When using the Rotor-Gene instrument, **Gain** needs to be set for all channels, so that the basic fluorescence is within **5–10 RFU**. **Attention** – in case of setting the Gain before the analysis, the temperature of Gain optimisation **must not exceed 42 °C**.
- Fluorescence acquisition must be set active for the FAM/SYBR, HEX/JOE/VIC, ROX and Cy5 channels.
- Instructions for setting up a cycler can be found at: https://www.generi-biotech.com – see the "download" section, or follow the instrument manufacturer's instructions for use.



DATA ANALYSIS

Determine the SARS-CoV-2 presence in a sample by analysing Ct values obtained with the software of your real-time PCR cycler. The limit of detection (LOD)* for this kit is 4 cop/rxn for RdRP gene, 4,6 cop/rxn for mutation A570D and 52,6 cop/rxn for mutation E484K.

*LOD has been set at a 95% confidence interval

Read the signal as Ct (threshold cycle) in a given fluorescence channel in the quantitation mode. Read Ct values **for viral genes in FAM, HEX and ROX channels, Ct values for exogenous positive control in the Cy5 channel**. For strongly positive samples, the exogenous positive control may not be amplified. For all active channels, **the same threshold fluorescence value (threshold)** must be set for reading. Follow the instructions of the cycler manufacturer.

First verify the analysis validity. Display signals of control samples.

Analysis validity

Analysis is considered valid when control signals correspond with the following layout:

- Positive Control CoV-2 SA – signals in the FAM/SYBR, ROX, and Cy5 channels
- Deionized Water as negative isolation control – a signal in the Cy5 channel
- Deionized Water as NTC – no signal

If the analysis is valid, continue with evaluation of the samples. Otherwise follow recommendations given in the Troubleshooting chapter.

Interpretation of results

The final outcome of the analysis is the evaluation of the SARS-CoV-2 RNA presence (i.e., sample positivity) and presence of mutations A570D and E484K.

In the first stage of analysis determine a presence of SARS-CoV-2 based on evaluation of signals from ROX and Cy5 channels:

VALID result	**ROX**	**Cy5**
Positive	**+**	**+/-**
Negative	**-**	**+**
Low positivity *	**Ct ⩾ 35**	**+**

INVALID result	**ROX**	**Cy5**
Failure of isolation or inhibition of RT-PCR, repeat analysis	**-**	**-**

* Sample with low positivity must have visible signal of exogenous positive control in Cy5 channel, otherwise the result is invalid.

At positive samples determine a presence of mutations based on signals in channels FAM, HEX and ROX. The signal in the ROX channel serves as a reference signal. The presence of mutations is determined according to signals in FAM and HEX channels. **The positive signal in the FAM or HEX channel is represented by an amplification curve that is shifted by a maximum of 7 cycles to the right of the curve in the ROX channel. The negative signal either does not contain an amplification curve at all, or the curve is shifted to the right by more than 7 cycles from the ROX signal.** The assessment based on Ct values is possible if the threshold fluorescence in all channels is set to the same level.

	FAM	**HEX**	**ROX**
E484K positive B.1.351 (501Y.V2, South African) P.1 (501Y.V3, Brazilian)	**Ct_{FAM} < Ct_{ROX}+7**	**-**	**+**
A570D positive B.1.1.7 (501Y.V1, UK)	**-**	**Ct_{HEX} < Ct_{ROX}+7**	**+**
A570D and E484K positive B.1.1.7 (501Y.V1, UK) with E484K	**Ct_{FAM} < Ct_{ROX}+7**	**Ct_{HEX} < Ct_{ROX}+7**	**+**
A570D and E484K negative	**-**	**-**	**+**

The evaluation table CoV2_A570D_E484K (name of the file CoV2_A570D_E484K_vN_N.xltx) can also be used to evaluate the results. The table is available on the manufacturer's website.

The evaluation table is for the Microsoft Excel 2010 and later version.

TROUBLESHOOTING

Test results can be considered correct only if the instructions indicated in the enclosed manual are followed. Where control samples give incorrect results, check the following:

- the expiry date of the kit
- the storage and manipulation conditions
- the pipette and cycler settings

Finding:	**Corrective action suggestion:**
A FAM, HEX or ROX signal is detected in the negative control (Deionized Water) reaction.	Reactions were most probably contaminated with a template. Repeat the analysis.
Standard Positive Control is not detected or is detected only in one/two of the channels.	A pipetting error probably occurred. Repeat the analysis.
A signal in the FAM, HEX or ROX channel is detected in the negative isolation control; however, no signal is detected in the PCR negative control (NTC).	A cross-contamination between samples probably occurred. Prepare new RNA isolates.
No signal in Cy5 channel is detected in the negative isolation control.	EPC Template RNA was probably not added in the isolation reactions or a failure of the isolation procedure occurred. Repeat the analysis following the Instructions for use chapter.
No signal in any channel is detected for the examined sample, though the analysis was evaluated as valid.	The inhibition of PCR probably caused a failure of the analysis. Perform an analysis with a new RNA isolate.

CONTENTS AND DESCRIPTION OF KIT COMPONENTS

Component [1]	Volume	Qty [2]	Concentration
Assay CoV-2 SA/BR, UK	0,5 ml [3]	1 | 5	4×
Master Mix OneStep Multi	1,0 ml [3]	1 | 5	2×
Positive Control CoV-2 SA	0,2 ml	1 | 1	4×
EPC Template RNA	1,0 ml	1 | 5	
Deionized Water	1,0 ml	1 | 1	

[1] Tube lid colour corresponds with reagent type.
[2] Number for kit size of 100 or 500 reactions.
[3] Volume equates to 100 PCR reactions of 20 µl of volume.

Assay CoV-2 SA/BR, UK

Assay CoV-2 SA/BR, UK is a mixture of amplification primers and fluorescently labelled probes. The probes allow the detection of **viral gene RdRP in the ROX channel** ($\lambda_{EXCITATION}$ = 575 nm, $\lambda_{EMISSION}$ = 602 nm and **exogenous positive control (EPC) in the Cy5 channel** ($\lambda_{EXCITATION}$ = 650 nm, $\lambda_{EMISSION}$ = 670 nm). **Mutation E484K in viral gene S in channel FAM** ($\lambda_{EXCITATION}$ = 495 nm, $\lambda_{EMISSION}$ = 520 nm) a **mutation A570D in viral gene S in channel HEX** ($\lambda_{EXCITATION}$ = 535 nm, $\lambda_{EMISSION}$ = 556 nm). Assay CoV-2 SA/BR, UK is supplied in a micro tube with a green cap. Mixing with Master Mix OneStep Multi provides a complete ready-to-use assay.

Master Mix OneStep Multi

Master Mix OneStep in the blue cap micro tube is an optimized mixture of buffer, reverse transcriptase, polymerase and nucleotides that is necessary for RT-qPCR.

Positive Control CoV-2 SA

Positive Control CoV-2 SA serves as a positive control (a standard) for a verification of the analysis validity. Positive control is positive in the channel ROX (CoV-2 RdRP), Cy5 (EPC) and FAM (CoV-2 E484K). It is supplied in a micro tube with a red cap. Handle Positive Control to avoid cross-contamination with other kit components and analysed samples.

EPC Template RNA

EPC Template RNA is an exogenous positive control for verification of the isolation process. It is supplied in a micro tube with a yellow cap.

Deionized Water

Deionized water serves as a negative isolation control and no-template control (NTC) in PCR. It is supplied in a tube with a transparent lid.

Reagents and equipment not included in the kit

- kit or reagents for the isolation of viral RNA
- single-use plastic micro tubes, strips or plates convenient for use in a PCR cycler
- adjustable micropipettes with the corresponding range
- disposable pipette tips with filters
- laboratory vortex and centrifuge
- real-time PCR cycler with software

WARNINGS AND PRECAUTIONS

Storage and manipulation conditions

- Store all kit components at a temperature below **-20 °C**.
- Assay is photosensitive; therefore, limit its handling in the light to the shortest time possible.
- Reagents are designed for work at laboratory temperature.
- Individual kit components may be repeatedly thawed and frozen **5 times at the most**. Do not freeze the complete assay resulting from mixing the Master Mix OneStep Multi and Assay CoV-2 SA/BR, UK. The final reaction mixture is **disposable**.
- If the above-mentioned conditions are followed, the kit is stable until its expiry date stated on the **box label**.

Safety measures

- The kit is designed for professional use only.
- When working with RT-qPCR reagents and material, always wear laboratory clothing and safety gloves.
- In case of skin or eye contact with reagents, rinse the affected area under running water.

Instructions for use

- Always use the enclosed version of the manual. The corresponding version number is marked on the label inside the box.
- Inappropriate reagents handling or adjustments of the workflow may negatively influence results and thus it is necessary to strictly follow the pipetting volumes, incubation times and temperature conditions as stated in the manual.
- Adhere to the expiry date of the kit indicated on the box label.
- Do not combine components from different batches of the kit.
- If any of the kit components is damaged upon receipt, do not use it and contact the manufacturer immediately. Keep the component for the purposes of an eventual claim.
- Use calibrated pipettes and instruments.
- Dispose of all waste material in accordance with the applicable legislation. The outer packing is made from paper, the inner segment from polyurethane and the micro tubes from polypropylene. Reagents may be handled as common waste. Dispose of the final PCR analysis product taking into account the risk of work space contamination.

Contamination precautions

- Assign specific spaces, equipment, material and protective equipment for the isolation of RNA/DNA from clinical material, and different ones for preparing RT-qPCR.
- Change your gloves and protective clothing whenever you suspect contamination.
- Never open an amplified PCR product in the place where the PCR reactions are prepared.
- Leave reagents open only for the time necessary to prepare PCR reactions.
- Use tips with filters when pipetting.
- When preparing a reaction mixture, take care not to contaminate any other component of the kit, or other samples, with the positive control. This may be avoided by closing all the micro tubes before manipulating a positive control.
- Use ultra-clean water for sample dilution; the Deionized Water provided with the kit may be used for this purpose.

SYMBOLS USED ON STICKERS

LOT	Batch code
	Expiry date
	Store at recommended temperature
Cont.	Contains
	Manufacturer
	Number of tests

PRODUCT LINE

gb SARS-CoV-2 Multiplex	Cat. no. 3231
gb SARS-CoV-2 Multiplex EndoC	Cat. no. 3234

REFERENCES

When using the kit, follow the manufacturer's manual for the cycler. The list of cyclers on which the kit´s performance parameters have been tested is available at the manufacturer's website.

For additional information please contact us at our e-mail address: info@generi-biotech.com or by phone: +420 495 056 314. Further information can also be found on our website www.generi-biotech.com.

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