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For research use only

SARS-CoV-2 Variants
Multiplex REAL-TIME PCR Detection Kit

Package S

REF **R3-P443-S3/9EU**

General information

Intended use:
SARS-CoV-2 Variants Multiplex REAL-TIME PCR Detection Kit is intended for detection of the S-gene mutations of the SARS-CoV-2 by Real-Time PCR method.
SARS-CoV-2 Variants Multiplex REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:
Multiplex Real-Time PCR with Reverse Transcription (RT-PCR), qualitative analysis.

Samples:
Nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

RNA extraction:
Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA-S DNA/RNA Extraction Kit** are recommended).

Features:

Multiplex analysis gives the opportunity of several NA targets detection in the same tube.
We recommend including in assay the negative control (C-) which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:
The automatic analysis for **SARS-CoV-2 Variants Multiplex REAL-TIME PCR Detection Kit** is available on "DNA-Technology" made DTprime[1] REAL-TIME Thermal Cyclers; the latest version of the software is available for download at https://www.dna-technology.com/software.

Time of analysis (including sample preparation procedure):
from 2.5 hours.

The number of tests:
96 (including one positive control and one negative control in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
1. Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	15 µL in each	12 8-tubes strips
2. Enzyme Taq/RT	Colorless transparent viscous liquid	55 µL	1 tube
3. SARS-CoV-2 Variants RT-PCR-buffer	Colorless transparent liquid	810 µL	2 tubes
4. Positive control	Colorless transparent liquid	130 µL	1 tube
Associated accessories:			
Strip's caps			12 8-caps

[1] - supported by 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
SARS-CoV-2, E-gene	S: 21765-21770 deletion (HV 69-70 DEL)	S: A23063T (N501Y)	S: G23012A (E484K)	S: G22813T (K417N)

Procedure

1 RNA extraction

⚠ Independently of DNA/RNA extraction kit used, a negative control sample should go through all stages of RNA extraction simultaneously with the RNA extraction from clinical samples.

Physiological saline solution can be used as a negative control sample in volumes as indicated in the instructions for use of extraction kits or negative control sample that is included in the corresponding extraction kit.

1.1 Mark the required number of the 1.5 mL plastic tubes considering samples and negative control (C-).

1.2 Perform the RNA extraction procedure according to user manual supplied with the **PREP-NA** and **PREP-NA-S** extraction kits.

Use RNase and DNase free pipette tips only.

The precipitate should be dissolved in 50 µL of the Dilution Buffer after drying. DNA-IC and RNA-IC from the **PREP-NA** kit are not used.

⚠ The resulting RNA preparation should be used for RT-PCR within two hours after preparation. If it is needed, the resulting RNA preparation can be stored at temperatures from minus 18 °C to minus 22 °C for no longer than a week with a single defrosting before reverse transcription.

2 PCR with Reverse Transcription (RT-PCR)

⚠ The reagents and tubes should be kept away from direct sun light!

Strictly observe the completeness of the strips and caps to them. Do not use the caps to the strips of the other kits!

2.1 Mark the required number of the tubes with paraffin sealed PCR-mix according to the number of samples to be analyzed, 1 tube for negative control (C-) and 1 tube for positive control (C+).

Example. If you need to test 2 samples, mark 4 tubes (one for each sample, one for "C-", one for "C+").

2.2 Vortex the SARS-CoV-2 Variants RT-PCR-buffer and Enzyme Taq/RT thoroughly for 3-5 s, then spin briefly for 1-3 s.

⚠ Enzyme Taq/RT should be got out from the freezer immediately prior to use.

2.3 Prepare the mixture of SARS-CoV-2 Variants RT-PCR-buffer and Enzyme Taq/RT. Add to the one tube:

- 15 x (N+1) µL of SARS-CoV-2 Variants RT-PCR-buffer;
- 0.5 x (N+1) µL of Enzyme Taq/RT,

N – is a quantity of the samples to be tested taking to account "C-", "C+".

2.4 Vortex the tube thoroughly. Then spin briefly for 1-3 s.

⚠ Mixture of SARS-CoV-2 Variants RT-PCR-buffer and Enzyme Taq/RT must be prepared immediately prior to use and should be used within two hours after preparation. If it is needed, the prepared mixture can be stored at the temperatures from 2 °C to 8 °C.

2.5 Add 15 µL of the SARS-CoV-2 Variants RT-PCR-buffer and Enzyme Taq/RT mixture into each tube. Avoid paraffin layer break.

2.6 Vortex the tubes with samples and "C-" and "C+" for 3-5 s and spin down the drops by centrifuging on vortex mixer for 1-3 s.

⚠ Close the strip before proceeding to the next DNA sample (or control sample) to prevent contamination. Use filter tips. Close strips tightly.

2.7 Add 10 µL of the RNA sample into corresponding tube. Do not add RNA into the "C-", "C+" tubes.

2.8 Add 10 µL of negative control sample (C-), which passed whole RNA extraction procedures into corresponding tube. Add 10 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

2.9 Spin strips for 3-5 s to collect drops.

2.10 Set the strips to real-time PCR thermal cycler.

2.11 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 2.10) and run PCR.

3 Data collection and data analysis

Registration of the PCR results is held in automatic mode. Interpretation of the PCR results should be performed according to the Table 1.

Table 1. Results interpretations

Detection channel					**Interpretation**
Fam	Hex	Rox	Cy5	Cy5.5	
Analyzed samples					
Cp ≤35	Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	**RNA of SARS-CoV-2 is detected** S-gene mutations of the SARS-CoV-2 is not detected
Cp ≤35	**For one or more Detection channels Cp is specified**				**One or more mutations of the S-gene is detected**
Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Unreliable result. Repeat PCR amplification or NA extraction or re-collect of a clinical sample, performed sequentially
Positive control sample					
Cp is specified	**Cp is specified**	**Cp is specified**	**Cp is specified**	**Cp is specified**	Positive result The results are valid
Negative control sample					
Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Negative result The results are valid

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Storage, shipping and handling requirements

The Enzyme Taq/RT should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The strips with paraffin sealed RT-PCR-Mix, SARS-CoV-2 Variants RT-PCR-buffer and positive control sample should be stored at the temperatures from 2 °C to 8 °C during the storage period.

Excessive temperature and light can be detrimental to product performance.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the Enzyme Taq/RT, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the Enzyme Taq/RT in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life – 12 months if all the conditions of transportation, storage and operation are met.

Contact our customer service department regarding quality issues with the kit:

8 800 200-75-15 (toll-free call for Russia)

+7 (495) 640-16-93 (chargeable call for CIS and foreign countries)

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Key to symbols

	Temperature limit		Consult instructions for use	REF	Catalogue number
	Use-by date		Manufacturer	LOT	Batch code
	Date of manufacture		Contains sufficient for <n> tests		Keep away from sunlight
	Caution	NON STERILE	Non-sterile		Do not reuse