DNA-TECHNOLOGY

1313 2025-06-21

RUO

For research use only

SARS-CoV-2 Lite REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

REF R3-P446-S3/9ER

VER 1313.2025.06.21

TABLE OF CONTENTS

1. INTENDED USE

The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** is designed to detect coronavirus SARS-CoV-2 in human biological samples (nasopharyngeal smears, oropharyngeal smears) with an aid reverse transcription (RT) and of polymerase chain reaction (PCR) methods.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit.**

The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The implemented method of reverse transcription followed by polymerase chain reaction is based on RNA reverse transcription process and subsequent amplification of cDNA.

The principle of the method is based on the use of the process of reverse transcription of RNA and subsequent amplification of cDNA, which consists of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent extension of polynucleotide chains from these primers with Taq polymerase.

In this kit, the "direct" RT-PCR technology is used for SARC-CoV-2 RNA detection without RNA isolation from biological samples.

The stages of RNA reverse transcription and cDNA PCR-amplification are carried out in one tube.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains dual target-specific probes bearing reporter fluorescent dyes (Fam, Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** contains the Internal control RNA-IC, which is intended to assess the quality of the RNA extraction and polymerase chain reaction.

DNA probe used for the detection of the SARS-CoV-2 (E, RdRp-genes) cDNA products is labeled with fluorescent reporter dye Fam. DNA probe used for the detection of the internal control (RNA-IC) amplification product is labeled with the fluorescent reporter dye Hex. The application of the dual fluorescent dyes allows detection of SARS-CoV-2-specific targets and the Internal control simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex		Rox	Cy5	Cy5.5
SARS-CoV-2 E gene, RdRp gene	IC*		-	-	-

* - Internal control RNA-IC

The automatic analysis for **SARS-CoV-2 Lite REAL-TIME-PCR Detection Kit** is available on "DNA-Technology" made DTlite[1] and DTprime[2] real-time thermal cyclers; the latest version of the software is available for download at https://www.dna-technology.com/software.

The **SARS-CoV-2 Lite REAL-TIME-PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) and Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

For the use of detecting thermal cyclers other than those listed above, please consult the reagent kit manufacturer for consultation.

3. CONTENT

Table 2. The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** content, package S (standard)

Reagent	Description	Nominal volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	2400 µL (25 µL in each tube)	12 8-tube strips
RT-PCR-buffer	Colorless transparent liquid	1.62 mL	1 tube
Enzyme Taq/RT	Colorless transparent viscous liquid	110 µL	1 tube
Internal control RNA-IC	Colorless transparent liquid	110 µL	1 tube
Positive control*	Colorless transparent liquid	130 µL	1 tube
Strip's caps	12 8-caps		

* - marking as C+ is allowed

All components are ready to use and do not require additional preparation for operation.

The kit is designed for 96 tests (including one positive control and one negative control in each run). It is recommended to perform no more than 12 runs.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single use sterile containers to collect biological material;
- Sterile tubes containing transport medium: "DNA-Technology" made **STOR-F** (REF P-901-1/1EU) or physiological saline solution (Sterile).

4.2. RNA extraction (optional) and PCR

- Biological safety cabinet class II;
- UV PCR cabinet;
- Refrigerator;
- Vortex mixer with rotor for strips;
- High speed centrifuge (RCF(g) 12000 - 16000);

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.

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

- Solid-state thermostat (temperature range 40-95 °C);
- Tube rack for strips;
- Tube rack for 1.5 mL tubes;
- 1.5 mL microcentrifuge tubes with caps;
- Single channel pipettes (dispensers covering 0.2-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from

https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

5.1. Storage conditions

- All components of the reagent kit, except the Enzyme Taq/RT must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- Paraffin sealed PCR-mix must be kept away from light.
- Enzyme Taq/RT must be stored in a freezer at the temperature from minus 22 °C to minus 18 °C throughout the shelf life of the kit.

WARNING! The excessive temperature and light can be detrimental to product performance.

5.2. Transport conditions

Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the kit components.

- It is allowed to transport the kit, except for Enzyme Taq/RT, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport Enzyme Taq/RT in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container up to 25 °C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

- All components of the kit, except for Enzyme Taq/RT, must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.
- PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light over the storage period.

- Enzyme Taq/RT must be stored in a freezer at temperatures from minus 22 °C to minus 18 °C over the storage period.

WARNING! The kits stored under undue regime must not be used.

An expired kit must not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

ATTENTION! The SARS-CoV-2 is classified as particularly pathogenic. Laboratories performing research on the detection of SARS-CoV-2 RNA are required to ensure the safety of work in accordance with the requirements of national legislation in the field of sanitary and epidemiological welfare.

Only specially trained personnel with corresponding education who have been trained at licensed courses of primary specialization in working with pathogenic microorganisms and who have received additional special training at advanced training courses on molecular and biological methods of analysis are allowed to work with the kit of reagents.

Handle and dispose all biological samples, reagents and materials used to carry out the analysis as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the analysis. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.

RNA extraction should be performed in biological safety cabinet class II-III with laminar flow. Preparation of reagents for RT-PCR can be performed in UV PCR cabinet.

In case of using “direct” PCR without RNA extraction, preparation of reagents for RT-PCR should be performed in biological safety cabinet class II-III laminar flow.

All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents’ appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current instruction.

7. SAMPLES

The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** is designed to detect Coronavirus SARS-CoV-2 in human biological samples (nasopharyngeal smears, oropharyngeal smears).

General requirements

Sampling procedure is carried out using special sterile disposable instruments – dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts, depending on the source of biological material in accordance with established procedures.

The features of biomaterial sampling

Each sample of biomaterial should be placed in a separate transport container in accordance with requirements in the table of guidance.

Interfering Substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

Interfering substances that may be present in a sample of biological material include the following endogenous and exogenous interfering substances: whole blood, mucus (mucin), and local drugs.
Whole blood above 5% v / v and guanidine isothiocyanate above 1% v / v influence the test results.
Mucus (mucin) at a concentration up to 10% mg / mL did not affect direct RT-PCR detection.
Chlorhexidine bigluconate 0.05%, Tantum Verde (benzydamine hydrochloride), Tizin (xylometazoline), Grippferon (human recombinant interferon alpha-2b) at a concentration of 5.0% v / v did not affect direct RT-PCR detection.
Hexoral (Hexetidine) and Listerine (Sodium Lauryl Sulfate) at a concentration above 0% may totally inhibit direct RT-PCR detection.
To reduce the amount of interfering substances that inhibit PCR, it is necessary to exclude the use of local drugs 6-12 hours before taking the biological material, to follow the rules for taking, transporting and storing it. Before taking biological material, it is not recommended to carry out hygienic procedures of the nasopharynx and oropharynx (including the use of toothpaste and rinses).

If you suspect the presence of a large amount of PCR inhibitors in the sample, it is recommended to use the nucleic acid isolation step.

ATTENTION! The use of a transport medium containing guanidine salts is prohibited.

To avoid RT-PCR inhibition, it is recommended to use "DNA-Technology" made **STOR-F** (REF P-901-1/1EU) or physiological saline solution (sterile) with **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit.**

Sample collection

Nasopharyngeal smears sampling

Take the smear with a dry sterile disposable swab into 1.5 mL plastic tubes with 300 mL of transport medium or physiological saline solution.

Order of taking:

1. Insert the swab carefully along the outer wall of the nose to a depth of 2-3 cm to the lower shell. Then lower the swab down slightly, insert into the lower nasal passage under the lower nasal conch, after a rotational movement remove along the outer wall of the nose.
2. Open the tube.
3. Put the swab into the tube with solution, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the swab from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used swab.
5. Close the tube tightly and mark it.

Oropharyngeal smears sampling

Take the smears with a dry sterile disposable swab into 1.5 mL plastic tubes with 300 mL of transport medium or physiological saline solution.

Order of taking:

1. Take the smear with a swab with a rotational movement from the surface of the tonsils, palatine arches and the back wall of the pharynx.
2. Open the tube.
3. Put the swab into the tube with solution, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the swab from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used swab.

5. Close the tube tightly and mark it.

Transportation and storage of the samples in accordance with guidance

Type of sample	Collecting material requirements	Transportation	Storage conditions before testing	Comments
Nasopharyngeal and oropharyngeal smears	Plastic test tubes and tampons smears**	4 °C	≤5 days: 4 °C >5 days*: minus 70 °C	Nasopharyngeal and oropharyngeal smears should be placed together in one tube to provide detection even in case of low viral load

* if it is not possible to store samples at minus 70 °C, store samples at minus 20 °C.

** to transport samples, use a transport medium for storing and transporting respiratory smears or physiological saline solution (if transportation to the laboratory is no more than 24 hours after taking the sample).

ATTENTION! Avoid repeated freezing and thawing of samples.

It is recommended to centrifuge the tube with biomaterial in transport medium at RCF(g) 12000 – 16000 for 1 minute before the analysis.

8. PROCEDURE

ATTENTION! The range of SARS-CoV-2 viral load can vary widely. In laboratory practice the risk of cross-contamination between samples remains serious danger, especially during aliquoting and RNA extracting (optional). Cross-contamination with high-copy nucleic acids can lead to sporadic false-positive results.

To prevent cross-contamination in the laboratory, the following rules are recommended:

- it is necessary to conduct a visual assessment of the incoming biomaterial and cull test tubes with broken integrity;
- if possible, it is recommended to analyze samples with symptoms of acute infection separately from the rest of the samples (the biological material for screening exposed individuals). It is desirable to work with the supposed high-copy samples in a separate box or after working with the supposed low-copy samples;
- it is necessary to use negative controls, starting from the stage of extracting RNA in each protocol;
- use tips with aerosol filters at all stages of the analysis;
- strictly follow the analysis, open the Eppendorf test tubes with tweezers or a special opener (do not touch inside the tube cap by the gloved hand); when applying reagents, do not touch inside the test tube by the tip (if this happened, immediately replace the tip).

In this kit, the “direct” RT-PCR technology is used: SARC-CoV-2 RNA detection is carried out without RNA isolation from test samples.

ATTENTION! For "direct" RT-PCR (without RNA isolation), the use of a transport medium containing guanidine salts is prohibited.

To avoid RT-PCR inhibition, it is recommended to use “DNA-Technology” made **STOR-F** (REF P-901-1/1EU) or physiological saline solution with **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit.**

To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose NA extraction methods that allow to remove PCR inhibitors.

8.1 PCR with Reverse Transcription (RT-PCR)

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

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

8.1.1 Mark the required number of the tubes or strips 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: to test 6 samples, mark 6 tubes (one for each sample), one for "C-" and one for "C+". The resulting number of tubes is 8.

8.1.2 Vortex the RT-PCR-buffer, Enzyme Taq/RT and RNA-IC thoroughly for 3-5 seconds, then spin for 1-3 seconds.

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

8.1.3 Prepare the mixture of RT-PCR-buffer, RNA-IC and Enzyme Taq/RT. Add to the one tube:

- 15 x (N+1) µL of RT-PCR-buffer;
- 1.0 x (N+1) µL of RNA-IC;
- 1.0 x (N+1) µL of Enzyme Taq/RT,

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

Example: to test 6 samples, mark 8 tubes. Prepare the mixture of RT-PCR-buffer, RNA-IC and Enzyme Taq/RT for 9 (8+1) tubes. Mix 135 µL of RT-PCR-buffer, 9.0 µL of RNA-IC and 9.0 µL of Enzyme Taq/RT.

Table 3. The quantity of reagents for the mixture

Number of samples in PCR run	**Number of marked tubes, taking into account C+ and C-**	**Recommended volume of tube for preparing mixture**	**Volume of reagents in mixture**		
			RT-PCR-buffer, µL	RNA-IC, µL	Taq/RT, µL
6	8	1.5 mL	135	9	9
14	16	1.5 mL	255	17	17
22	24	1.5 mL	375	25	25
30	32	1.5 mL	495	33	33
38	40	1.5 mL	615	41	41
46	48	1.5 mL	735	49	49
54	56	1.5 mL	855	57	57
62	64	1.5 mL	975	65	65
70	72	1.5 mL	1095	73	73
78	80	1.5 mL	1215	81	81
86	88	2.0 mL	1335	89	89
94	96	2.0 mL	1455	97	97

ATTENTION! Taking the Enzyme Taq/RT, it is necessary to dip the tip no more than 1.0 mm and observe the rules for dosing viscous liquids. Thoroughly flush the remaining Enzyme Taq/RT from the tip by pipetting at least 5 times.

8.1.4 Vortex the tube with the mixture of RT-PCR-buffer, RNA-IC and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin for 1-3 seconds.

ATTENTION! Mixture of RT-PCR-buffer, RNA-IC and Enzyme Taq/RT must be prepared immediately prior to use and should be used within one hour after preparation. If it is needed, the prepared mixture can be stored at the temperatures from 2 °C to 8 °C but for no longer than one hour.

8.1.5 Add 15 µL of the RT-PCR-buffer, RNA-IC and Enzyme Taq/RT mixture into each tube. Avoid paraffin layer break. Close the strips.

8.1.6 Vortex the tubes with “C-” and “C+” for 3-5 seconds and spin down drops for 1-3 seconds. It is recommended to centrifuge the tubes with samples at RCF(g) 12000 – 16000 for 1 minute.

ATTENTION! Open the tube, add sample (or control), then close the strip before proceeding to the next sample to prevent contamination. Close the strips tightly. Use filter tips.

8.1.7 Add 10 µL of the NA sample or 10 µL of the sample, taken in transport medium, into corresponding tubes. Avoid paraffin layer break. Close the strips tightly.

8.1.8 Add 10 µL of negative control (C-), into corresponding tube. Add 10 µL of positive control (C+) into corresponding tube. Avoid paraffin layer break. Close the strips tightly.

8.1.9 Spin down the strips for 3-5 seconds to collect drops.

ATTENTION! RT-PCR must be carried out IMMEDIATELY AFTER preparing the reaction mixtures with added biomaterial samples. It is not allowed to store the tubes prepared for the analysis.

8.1.10 Set the strips into the real-time thermal cycler.

8.1.11 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID’s of the samples, positive and negative controls. Specify the position of the strips in the thermal unit (see 8.1.10) and run PCR. See table 4.

For use with CFX96 and Applied Biosystems Quant Studio 5 thermal cyclers consult user manual for devices. See Tables 5 - 6.

Table 4. The PCR program for DTlite and DTprime thermal cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	60	5	0	1		Cycle
2	92	1	0	1		Cycle
3	92	0	10	5		Cycle
	58	0	10			
	67	0	10			
4	92	0	5	25		Cycle
	58	0	10		√	
	64	0	10			
5	90	0	5	15		Cycle
	58	0	10		√	
	64	0	10			
6	80	0	1	1		Cycle
7	10[1]	…	…	Holding		Holding
√ - optical measurement [1] – holding at 25°C is allowed						

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

Table 5. Amplification program for CFX96 thermal cycles

№ of block (Step)	Temperature, °C	Time min:sec	Number or cycles (repeats)
1	60	05:00	1
2	94	01:00	1
3	94	0:10	5
4	59	0:10	
5	67	0:10	
6	94	0:05	40
7	59 **√**	0:10	
8	64	0:10	
√- optical measurements (Plate Read), set measurement of fluorescence on Fam and Hex channels at 59 °C			

Table 6. Amplification program for Applied Biosystems Quant Studio 5 thermal cycles

Stage	Step no.	Temperature, °C	Time min:sec	Number of cycles (repeats)
Hold	1	60	05:00	1
	2	94	01:00	1
PCR stage	1	94	0:10	5
	2	59	0:10	
	3	67	0:10	
PCR stage	1	94	0:05	40
	2	59 **√**	0:10	
	3	64	0:10	
√- Fam fluorophores data collection, Hex included				

9. CONTROLS

The **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** contains positive control. Positive control is a cloned part of the virus genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The kit includes the Internal control RNA-IC. RNA-IC is intended to assess the quality of polymerase chain reaction. To reveal possible contamination a negative control is required.

ATTENTION! A negative control should go through all stages of RNA extraction. Physiological saline solution can be used as a negative control.

For **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** the test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not considered;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

For **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does not have positive result, it is necessary to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling requirements.

If negative control (C-) has positive result, all results of current PCR run are considered false. Decontamination is required.

10. DATA ANALYSIS

In case of using DNA-Technology made real-time PCR thermal cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

For CFX96 (Bio-Rad) detecting thermal cyclers, it is recommended to use the regression analysis type (Cq Determination Mode: Regression).

When using Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd.) thermal cyclers, "Relative Threshold" must be selected in the algorithm settings. In the Ct settings select the initial baseline cycle “1” and the final baseline cycle “40”.

Registration of the PCR results is held in automatic mode. Interpretation of the PCR results should be performed accordance to the Table 6.

Table 7. Interpretation of PCR results

Detection channel		**Result interpretation**
Fam	Hex	
Samples		
Cp/Cq/Ct ≤34	Is not considered	**SARS-CoV-2 coronavirus RNA is detected**
Cp/Cq/Ct >34	Is not considered	This result indicates a low content of the SARS-CoV-2 coronavirus RNA, which may be associated with a low viral load in a biological sample, with cross-contamination with high-copy samples, or with inhibition of RT-PCR. Re-sampling of biomaterial or repeated RT-PCR should be performed once. If the result is repeated, the final result should be "SARS-CoV-2 coronavirus RNA is detected".
Cp/Cq/Ct is not specified	**Cp/Cq/Ct is specified**	SARS-CoV-2 RNA is not detected
Cp/Cq/Ct is not specified	Cp/Cq/Ct is not specified	Invalid result
Positive control		
Cp/Cq/Ct is specified	Is not considered	Positive result. The results of PCR run are valid.
Negative control		
Cp/Cq/Ct is not specified	**Cp/Cq/Ct is specified**	Negative result. The results of PCR run are valid.

Unreliable result can be related to the presence of PCR inhibitors in DNA sample, incorrect analysis procedure, violation of the amplification temperature regimen, etc.

In case of suspicion of the presence of inhibitors in sample, it is necessary to conduct a method that includes the nucleic acid extraction from sample, or repeat sampling.

In case of suspicion of incorrect analysis procedure, it is recommended to repeat the reverse transcription and polymerase chain reaction step.

ATTENTION! A single negative test result, especially if it is a sample from the upper respiratory tract, does not exclude infection.

11. SPECIFICATIONS

a. The analytical specificity of the **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit** was assessed by bioinformatic analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

Since it is impossible to exclude the occurrence of new mutations in the genome of the SARS-CoV-2 coronavirus, two genome sites were selected as targets to improve the reliability of analysis: the RdRp and E genes sites.

In human biological samples containing RNA of the SARS-CoV-2 coronavirus, the detecting thermal cycler must detect a positive result of amplification of a specific product on Fam detection channel.

In human biological samples that do not contain SARS-CoV-2 coronavirus RNA, the detecting thermal cycler should record a positive result of amplification for the internal control on Hex detection channel and a negative result of the amplification of a specific product through on Fam detection channel.

There are not non-specific positive results of amplification of RNA sample in the presence Human Coronavirus HKU-1, Human Coronavirus NL-63, Human Coronavirus 229E, Human Coronavirus OC-43, Human Rhinovirus, Human Respiratory syncytial virus A, B, Human Parainfluenza virus type 1, Human Parainfluenza virus type 2, Human Parainfluenza virus type 3, Human Parainfluenza virus type 4, Human Metapneumovirus, MERS-CoV, DNA Human Adenovirus, MRSA, *Legionella pneumophila*, DNA *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Bordetella pertussis*, *Bordetella parapertussis*, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. Analytical sensitivity. LOD (limit of detection) is 10^3 genome equivalents per 1.0 mL of sample (nasopharyngeal smears, oropharyngeal smears in transport medium).

c. Diagnostic characteristics

Number of samples (n) - 290;

Diagnostic sensitivity (95% CI) - 100% (94.94-100%);

Diagnostic specificity (95% CI) – 100% (98.33-100%).

12. TROUBLESHOOTING

Table 8. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **SARS-CoV-2 Lite REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.com

14. KEY TO SYMBOLS

RUO	For research use only		Manufacturer
	Temperature limit		Date of manufacture
	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date	REF	Catalogue number
LOT	Batch code		Keep away from sunlight
VER	Version		Do not reuse
	Caution		

REF R3-P446-S3/9ER VER 1313.2025.06.21