DNA-TECHNOLOGY

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CE IVD

For professional use only

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

INSTRUCTION FOR USE

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"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

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1. INTENDED USE

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit i**s intended for research and diagnostic applications. The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit i**s an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit i**s designed to detect SARS-CoV-2 (COVID-19 virus, 2019-nCoV) and SARS-like coronaviruses in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: nasopharyngeal smears, oropharyngeal smears, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

Indications for the use:

- persons with ARVI symptoms and who have been in contact with COVID-19 infected, regardless of their age;
- persons of all ages without ARVI symptoms (in the centers of infection/ in the conditions of infection spread) for the purpose of early detection of coronavirus to prevent further spread of infection.

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

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice. The safety of laboratories should be ensured in accordance with the requirements of legislation in the field of sanitary and epidemiological welfare.

Potential users: personnel qualified in molecular diagnostics methods and in working with pathogenic microorganisms and working in the clinical and diagnostic 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 RNA reverse transcription stage and PCR amplification of cDNA stage are performed in one test 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 (package S) or Taq-polymerase blocked by antibodies (packages A, N). The polymerase chain reaction starts only when paraffin melts or after the Taq-polymerase/antibody complex temperature dissociating, which excludes the possibility of non-specific annealing of primers on the DNA target during the initial warm-up of the test tube.

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** includes the Internal control (RNA-IC “A”), which is intended to assess the quality of the RNA extraction and polymerase chain reaction.

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains four target-specific probes bearing reporter fluorescent dyes (Fam, Hex, Rox and Cy5) 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.

DNA probe used for the detection of the SARS-CoV-like coronaviruses product amplification includes fluorescent dye Fam. DNA probe used for the detection of the SARS-CoV-2 (E-gene) product amplification includes fluorescent dye Rox. DNA probe used for the detection of the SARS-CoV-2 (N-gene) product amplification includes fluorescent dye Cy5. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of four fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red
SARS-CoV-like Coronaviruses	IC*	SARS-CoV-2 coronavirus, E-gene	SARS-CoV-2 coronavirus, N-gene

*- Internal control (RNA-IC "A")

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** is also approved for use with Rotor-Gene Q (Qiagen) and CFX96 (Bio-Rad) real-time thermal cyclers.

3. CONTENT

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** content is represented in Tables 2-5.

Table 2. The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** content, package S (standard) for R3-P436-23/9EU and R3-P436-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	1440 µL (15 µL in each tube)	96 tubes or 12 8-tube strips
RT-PCR-buffer	Colorless transparent liquid	1620 µL (810 µL in each tube)	2 tubes
Enzyme Taq/RT	Colorless transparent viscous liquid	55 µL	1 tube
Internal control RNA-IC "A"	Colorless transparent liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps [1]	12 8-caps		

Table 3. The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** content, package A (strips) for R3-P436-XA/XEU

Reagent	Description	Total volume	Amount
PCR-mix Stream	Slightly pink transparent liquid	2304 µL (72 µL in each tube)	4 8-tube strips
RT-PCR-buffer Stream	Colorless transparent liquid	2.4 mL (1.2 mL in each tube)	2 tubes
Enzyme Taq/RT	Colorless transparent viscous liquid	200 µL (100 µL in each tube)	2 tubes
Internal control RNA-IC "A"	Colorless transparent liquid	4.0 mL (1.0 mL in each tube)	4 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

[1] - for detection kit packaged in strips REF R3-P436-S3/9EU

Table 4. The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** content, package A (tubes) for R3-P436-VA/XEU

Reagent	Description	Total volume	Amount
RT-PCR-mix Stream	Slightly pink transparent liquid	4.8 mL (1.2 mL in each tube)	4 tubes
Enzyme Taq/RT	Colorless transparent viscous liquid	200 µL (100 µL in each tube)	2 tubes
Internal control RNA-IC "A"	Colorless transparent liquid	4.0 mL (1.0 mL in each tube)	4 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

Table 5. The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** content, package N for R3-P436-N3/9EU

Reagent	Description	Total volume	Amount
RT-PCR-mix	Slightly pink transparent liquid	1.3 mL	1 tube
Enzyme Taq/RT	Colorless transparent viscous liquid	55 µL	1 tube
Internal control RNA-IC "A"	Colorless transparent liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

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

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (packages S and N) (it is not recommended to carry out more than 12 performances) and 384 tests (package A) (one performance for 384 samples or two performances for 192 samples each), including the analysis of the unknown samples, positive control and negative control.

ATTENTION! It is not recommended to perform less than 8 samples (6 defined samples, one positive control and one negative control) in one run. It can lead to situation when the volume of enzyme will be insufficient.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single use sterile containers to collect clinical material;
- Sterile tubes containing transport medium: "DNA-Technology" made **STOR-F** (REF P-901-1/1EU) or equivalent for the transportation of the sample.

4.2. RNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II-III;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) 12000 – 16000);
- Solid-state thermostat (temperature range 24-65°C) or solid-state thermostat with timer ("DNA-Technology" made TT-2 "Thermit" thermostat or equivalent are recommended);

- Tube rack for 1.5 mL tubes;
- 1.5 mL microcentrifuge tubes with caps;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free filtered pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 0.2-1000 µL volume range);
- RNase and DNase free filtered pipette tips for semi-automatic pipettes (volume 20 µL, 200 µL, 1000 µL);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** REF P-002/1EU, **PREP-NA-S** REF P-007-N/1EU and **PREP-MB-NA** REF P-114-A/8EU, REF P-114-N/4EU DNA/RNA extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes or strips;
- Rotor for strips (if package in strips is used);
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- DTstream 12M1 or 15M1 dosing device (using detection kit in the package S, strips);
- DTstream 12M4 or 15M4 dosing device (using detection kit in the package A);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream dosing device;
- DTpack plate sealer ("DNA-Technology Research & Production", LLC) (using detection kit in the package A);
- Vortex mixer (RCF(g) 500) with microplate adapter (using detection kit in the package A);
- Polymer thermal film for 384-well microplate sealing (using detection kit in the package A);
- 384-well microplate (using detection kit in the package A);
- 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 http://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.

All components of **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**, except Enzyme Taq/RT, must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix and RT-PCR-mix must be stored out of light at temperatures from 2 °C to 8 °C during the storage period. The excessive temperature and light can be detrimental to product performance. The Enzyme Taq/RT must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

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 of the kit following the first opening of the primary container:

- components of the kit except Enzyme Taq/RT should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix and RT-PCR-mix should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- Enzyme Taq/RT should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kits stored under undue conditions should not be used.

An expired **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** should 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/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

ATTENTION! The SARS-CoV-2 coronavirus 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 medical or biological (veterinary) 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 diagnostics are allowed to work with the kit of reagents.

Handle and dispose all biological samples, reagents and materials used to carry out the assay 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 besterile, 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 assay. Wear powder-free surgical gloves and protective clothing (work clothes and personal protective equipment). Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC 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 can not 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. 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 special closed container containing a disinfectant solution. Do not open the tubes after amplification. Work surfaces, as well as rooms where PCR is performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. 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 manual.

7. SAMPLES

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** is designed to detect RNA extracted from the nasopharynx and oropharynx smears, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm, depending on professional prescription.

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.

PCR inhibitors are the presence of hemoglobin in the RNA sample as a result of incomplete removal during the extraction of RNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the RNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: 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 medicines, including those that are contained in nasal sprays, etc. should be removed during the NA extraction using sample preparation kits. 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 from the sample as much as possible. It is not recommended to use express methods of NA extraction.

The features of biomaterial sampling

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

Sample taking is made with special sterile single-use tools – probes, cytobrushes, and swabs depending on the source of biological material according to established procedure.

Transportation and storage of the samples

Type of the sample	Collecting material requirements	Transportation	Storage conditions before transportation	Comments
Nasopharynx and oropharynx smears	Plastic test tubes and probes for smears**	4 °C	≤5 days: 4 °C >5 days *: minus 70 °C	Nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load
Bronchoalveolar lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: minus 70 °C	A small sample dilution is possible
Endotracheal aspirate, nasopharyngeal aspirate or nasal lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: minus 70 °C	
Phlegm	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *: minus 70 °C	Make sure that the material is from the lower respiratory tract

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

** Use a transport medium for storage and transportation of the respiratory smears or saline solution (if transportation to the laboratory no more than 24 hours after taking the sample) or a dry probe (if transportation to the laboratory no more than 4 hours after taking the sample).

It is recommended to use transport media containing preservatives, for example, **STOR-F** produced by DNA-Technology or similar registered in the established order, intended for further study of samples by PCR.

ATTENTION! Avoid repeated freezing and thawing of samples.

Samples must be transported in accordance with the requirements of the sanitary legislation in relation to pathogenic microorganisms.

8. PROCEDURE

ATTENTION! The range of SARS-CoV-2 viral load can vary widely from very low values (10^4 or less copies/mL) in the biomaterial of asymptomatic carriers and patients in the recovery stage to extremely high values (more than 10^9 copies/mL) in the biomaterial of patients with a clinical picture of acute viral pneumonia. In this regard, when performing research in a clinical laboratory, the risk of cross-contamination between samples at all stages of work is a serious danger, especially during aliquoting and RNA extracting. Cross-contamination with high-copy biomaterial can lead to sporadic false-positive results.

To prevent cross-contamination of the biological material 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 of patients from a hospital with symptoms of acute infection separately from the rest of the samples (the biological material for screening exposed individuals and patients with mild disease). 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 control samples, starting from the stage of extracting RNA in each protocol;
- use tips with aerosol filters at all stages of the assay;

- strictly follow the assay procedure, open the Eppendorf test tubes with tweezers (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).

8.1 RNA extraction

For RNA extraction from the nasopharynx and oropharynx smears, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm, RNA extraction kits are used (see Table 6).

Table 6. The reagent kits validated for RNA extraction and further study with the **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit**

Reagent kit	Biomaterial
PREP-NA DNA/RNA Extraction Kit, produced by "DNA-Technology"	nasopharynx and oropharynx smears, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm (or nasopharynx and oropharynx smears if shortened method in accordance with Annex A is used)
PREP-NA-S DNA/RNA Extraction Kit, produced by "DNA-Technology"	nasopharynx and oropharynx smears
PREP-MB-NA DNA/RNA Extraction Kit (package A), produced by "DNA-Technology"	nasopharynx and oropharynx smears
AmpliSens® RIBO-prep, produced by InterLabService Ltd.	nasopharynx and oropharynx smears, bronchoalveolar lavage, endotracheal, nasopharyngeal aspirate, phlegm

RNA extraction is carried out according to the extraction kit instructions.

ATTENTION! The volume of the resulting RNA preparation should not exceed 50 µL.

ATTENTION! The resulting RNA preparation must be used immediately for RT-PCR. 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 defrost before reverse transcription.

8.2 The features of biomaterial preparation for SARS-CoV-2 coronavirus RNA testing

ATTENTION! Do not perform centrifugation as a pretreatment of nasopharyngeal and oropharyngeal smears taken into transport medium.

ATTENTION! For RNA extraction, 100 µL of the sample is used.

8.3 The use of control samples at the stage of nucleic acid extraction

8.3.1 Internal control sample

To exclude false negative results of the study and to control the quality of the study, it is necessary to use an internal control sample to the clinical samples at the stage of nucleic acid extraction.

The internal control (RNA-IC "A") from the **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** should be used as an internal control sample. The RNA-IC "A" is an artificial RNA packed in phage particle. It is irrelevant to SARS-CoV-2 and amplified with separate pair of primers and probe.

The RNA-IC "A" should be used in the amount of 10 µL per sample.

ATTENTION! The internal control (RNA-IC) and internal control (DNA-IC) from the **PREP-NA DNA/RNA Extraction Kit** are not used.

8.3.2 Negative control sample

To exclude false positive results of the study and to control the quality of the study, it is necessary to use a negative control sample from the nucleic acid extraction stage.

ATTENTION! Independently of DNA/RNA extraction kit used, a negative control sample should go through all stages of DNA/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 include in the corresponding extraction kit.

8.4 PCR with Reverse Transcription (RT-PCR) for package S

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

ATTENTION! When using package S (R3-P436-S3/9EU), strips, strictly observe the completeness of the strips and caps to them. Do not use the caps to the strips of the other kits!

8.4.1 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: 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.

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

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

8.4.3 Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT. Add to the one tube:

- 15.0 x (N+1) µL of 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+".

Example: to test 6 samples, mark 8 tubes. Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT for 9 (8+1) tubes. Mix 135 µL of RT-PCR-buffer and 4.5 µL of Enzyme Taq/RT.

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.

8.4.4 Vortex the tube with the mixture of RT-PCR-buffer and Enzyme Taq/RT thoroughly. Then spin briefly for 1-3 seconds.

ATTENTION! Mixture of RT-PCR-buffer 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.

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

8.4.6 Vortex the tubes with samples and "C-" and "C+" for 3-5 seconds and spin down the drops for 1-3 seconds.

ATTENTION! When using the **PREP-MB-NA DNA/RNA Extraction Kit** for RNA extraction, it is necessary to place the tubes with the RNA preparation into a magnetic tube rack after vortex. If, after extraction, the supernatant containing the extracted RNA was transferred into new test tubes, centrifugation after vortexing is performed on vortex-microcentrifuge for 3-5 seconds.

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

8.4.7 Add 10 µL of the RNA sample into corresponding tubes. Do not add RNA into the "C-", "C+" tubes. Avoid paraffin layer break.

8.4.8 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.

8.4.9 Spin down the tubes for 3–5 seconds to collect drops (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

8.4.10 Set the tubes/strips into the Real-time Thermal cycler.

8.4.11 Launch the operating software for DT instrument[2]. Add corresponding test[3], 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 8.4.10) and run PCR.

For use with Rotor-Gene Q and CFX96 (Bio-Rad) real-time thermal cyclers consult user manual for devices. See Tables 7, 8.

Table 7. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time, sec	Cycle repeats
Cycling	32 deg	1200	1 time
Cycling 2	95 deg	300	1 time
Cycling 3	94 deg	10	50 times
	60 deg √	15	

√ - optical measurement, set the fluorescence measurement (Acquiring) on the channels Green (Fam), Yellow (Hex), Orange (Rox) and Red (Cy5) at 60 °C

Table 8. The PCR program for CFX96 (Bio-Rad)

Step	Temperature, °C	Time, min:sec	Cycle repeats
1	35	20:00	1
2	95	5:00	1
3	94	0:15	50
4	64 √	0:20	

√ - optical measurement (Plate Read), set the fluorescence measurement on the Fam, Hex, Rox, and Cy5 channels at 64 °C

8.5 PCR with Reverse Transcription (RT-PCR) using DTStream (for package S, strips)

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!

8.5.1 Mark the required number of the 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.

8.5.2 Vortex the RT-PCR-buffer and Enzyme Taq/RT 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.

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

8.5.3 Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT. Follow the DTStream instructions or mix in a separate tube:

- 15 x (N+1) µL of 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+”.

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.

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

ATTENTION! Mixture of RT-PCR-buffer 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.

8.5.5 Vortex the tubes with samples and “C-” and “C+” for 3-5 seconds and spin down the drops for 1- 3 seconds.

8.5.6 Set tubes with mixture of RT-PCR-buffer and Enzyme Taq/RT, analyzed samples, positive and negative controls and strip tubes with PCR-mix to the DTstream and dispense the components according to the instruction manual.

8.5.7 Close the caps of the strips.

8.5.8 Spin the strips for 3-5 seconds in vortex.

8.5.9 Set the strips into the Real-time Thermal cycler.

8.5.10 Launch the operating software for DT instrument[4]. Add corresponding test[5], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 8.5.9) and run PCR.

For use with Rotor-Gene Q and CFX96 (Bio-Rad) real-time thermal cyclers consult user manual for devices. See Tables 7, 8.

8.6 PCR with Reverse Transcription (RT-PCR) using DTStream (for package A, strips)

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

8.6.1. Thoroughly mix the content of RT-PCR-buffer Stream and Enzyme Taq/RT tubes on a vortex mixer and vortex for 3-5 seconds. To carry out 384 detections, 2 tubes with RT-PCR-buffer Stream and 2 tubes with Enzyme Taq/RT are used simultaneously. To carry out 192 detections, 1 tube with RT-PCR-buffer Stream and 1 tube with Enzyme Taq/RT are used simultaneously.

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

8.6.2. Vortex the strips with PCR-mix Stream for 1-3 seconds. To carry out 384 detections, vortex 4 strips with PCR-mix Stream. To carry out 192 detections, vortex 2 strips with PCR-mix Stream.

8.6.3. Shake tubes with positive control for 3-5 seconds on vortex mixer, then vortex for 1-3 seconds.

8.6.4. Set the tubes or plates with RNA samples onto the DTstream workbench according to the setting protocol.

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

8.6.5. Set the strips with PCR-mix Stream, the tubes with RT-PCR-buffer Stream, Enzyme Taq/RT, positive control, and the new 384-well microplate onto the DTstream workbench.

8.6.6. Open the strips with PCR-mix Stream after carefully removing the protection seal and carry out the dosing of the components according to the user manual.

8.6.7. Place the 384-well microplate carefully, without shaking, into the DTpack plate sealer carrier after the program on DTstream is finished.

8.6.8. Seal the 384-well microplate with thermal film according to the DTpack user manual.

8.6.9. Vortex the 384-well microplate at RCF(g) 500 for 30 seconds.

8.6.10. Place the 384-well microplate into the detecting thermal cycler unit.

8.6.11. Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.6.10) and run PCR.

8.7 PCR with Reverse Transcription (RT-PCR) using DTStream (for package A, tube)

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

8.7.1. Thoroughly mix the content of RT-PCR mix Stream and Enzyme Taq/RT tubes on a vortex mixer and vortex for 3-5 seconds. To carry out 384 detections, 4 tubes with RT-PCR mix Stream and 2 tubes with Enzyme Taq/RT are used simultaneously. To carry out 192 detections, 2 tubes with RT-PCR mix Stream and 1 tube with Enzyme Taq/RT are used simultaneously.

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

8.7.2. Shake tubes with positive control for 3-5 seconds on vortex mixer, then vortex for 1-3 seconds.

8.7.3. Set the tubes or plates with RNA samples onto the DTstream workbench according to the setting protocol.

8.7.4. Set the tubes with RT-PCR mix Stream, Enzyme Taq/RT, positive control, and the new 384-well microplate onto the DTstream workbench and carry out the dosing of the components according to the user manual.

8.7.5. Place the 384-well microplate carefully, without shaking, into the DTpack plate sealer carrier after the program on DTstream is finished.

8.7.6. Seal the 384-well microplate with thermal film according to the DTpack user manual.

8.7.7. Vortex the 384-well microplate at RCF(g) 500 for 30 seconds.

8.7.8. Place the 384-well microplate into the detecting thermal cycler unit.

8.7.9. Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.7.8) and run PCR.

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

8.8 PCR with Reverse Transcription (RT-PCR) for package N

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

8.8.1. Mark the necessary amount of 0.2 mL amplification tubes or 0.2 mL strip tubes for each test sample, negative control (C-) and 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.

8.8.2. Vortex the RT-PCR-mix and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin briefly for 1-3 seconds.

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

8.8.3. Prepare the mixture of RT-PCR-mix and Enzyme Taq/RT. Add to the one tube:

- 12.0 x (N+1) µL of RT-PCR-mix;

- 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+".

Example: to test 6 samples, mark 8 tubes. Prepare the mixture of RT-PCR-mix and Enzyme Taq/RT for 9 (8+1) tubes. Mix 108 µL of RT-PCR-mix and 4.5 µL of Enzyme Taq/RT.

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.

8.8.4. Vortex the tube with the mixture of RT-PCR-mix and Enzyme Taq/RT thoroughly. Then spin briefly for 1-3 seconds.

ATTENTION! Mixture of RT-PCR-buffer 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.

8.8.5. Add 12 µL of the RT-PCR-mix and Enzyme Taq/RT mixture into each tube. Close the tubes/strips.

8.8.6. Vortex the tubes with samples and "C-" and "C+" for 3-5 seconds and spin briefly for 1-3 seconds.

ATTENTION! When using the **PREP-MB-NA DNA/RNA Extraction Kit** for RNA extraction, it is necessary to place the tubes with the RNA preparation into a magnetic tube rack after vortex. If, after extraction, the supernatant containing the extracted RNA was transferred into new test tubes, centrifugation after vortex is performed on vortex-microcentrifuge for 3-5 seconds.

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

8.8.7. Add 6.0 µL of the RNA sample into corresponding tubes. Do not add RNA into the "C-", "C+" tubes.

8.8.8. Add 6.0 µL of negative control sample (C-), which passed whole RNA extraction procedures into corresponding tube. Add 6.0 µL of positive control sample (C+) into corresponding tube.

8.8.9. Spin down the tube/strip for 3–5 seconds to collect drops (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

8.8.10. Set the tubes/strips to the real-time PCR thermal cycler.

8.8.11. Launch the operating software for DT instrument[8]. Add corresponding test[9], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.8.10) and run PCR.

For use with Rotor-Gene Q and CFX96 (Bio-Rad) real-time thermal cyclers consult user manual for devices. See Tables 7, 8.

9. CONTROLS

The **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** contains positive control sample. 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 "A"). RNA-IC "A" is intended to assess the quality of the RNA extraction and polymerase chain reaction. The RNA-IC "A" is an artificial RNA packed in phage particle. It is irrelevant to SARS-CoV-2 and amplified with separate pair of primers and probe. To reveal possible contamination a negative control is required.

ATTENTION! A negative control sample should go through all stages of RNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

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 taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control are not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

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

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.

The Real-time PCR Thermal Cyclers detects and interprets results automatically. Analysis will be performed by Real-Time PCR application. The interpretation should be performed in accordance with Tables 9, 10.

Table 9. The interpretation of assay results

Detection channel				**Interpretation**
Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	
Analyzed samples				
Cp/Ct/Cq is specified	Is not considered	**Cp/Ct/Cq is specified**	**Cp/Ct/Cq is specified**	**SARS-CoV-2* RNA is detected**
Cp/Ct/Cq is specified	Is not considered	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	**RNA of SARS-CoV-like coronaviruses is detected**, SARS-CoV-2 RNA is not detected
Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	RNA of SARS-CoV-like coronaviruses is not detected, SARS-CoV-2 RNA is not detected
Positive control sample				
Cp/Ct/Cq is specified	Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	**Cp/Ct/Cq is specified**	Positive result The results are valid
Negative control sample				
Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Negative result The results are valid

*Simultaneous presence of SARS-CoV-2 coronavirus and other coronaviruses like SARS-CoV in the RNA sample is possible

Table 10. Other possible results

Detection channel				**Interpretation**
Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	
Analyzed samples				
Cp/Ct/Cq ≤ 35	Is not considered	**Cp/Ct/Cq is specified**	Cp/Ct/Cq is not specified	Additional research is required, there is a possible mutation in one of the SARS-CoV-2 genes
		Cp/Ct/Cq is not specified	**Cp/Ct/Cq is specified**	
Cp/Ct/Cq ≥ 35	Is not considered	**Cp/Ct/Cq ≥ 35**	Cp/Ct/Cq is not specified	**SARS-CoV-2* RNA is detected**
Cp/Ct/Cq is not specified	Is not considered	Cp/Ct/Cq is not specified	**Cp/Ct/Cq ≥ 35**	
Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Cp/Ct/Cq is not specified	Unreliable results.

*Simultaneous presence of SARS-CoV-2 coronavirus and other coronaviruses like SARS-CoV in the RNA sample is possible

Other assay results are interpreted as “Unreliable results”.

Unreliable results may be caused by the presence of inhibitors in the nucleic acid preparation obtained from the clinical material, errors in the pre-analytical stage, incorrect implementation of the analysis Protocol,

non-compliance with the temperature mode of amplification, etc. In this case, either re-staging of reverse transcription and polymerase chain reaction, or re-extracting of the nucleic acid preparation, or re-collecting of clinical material (performed sequentially) is required.

When the expressed growing fluorescence (Cp/Ct/Cq is specified) on the Fam/Green, Rox/Orange, or Cy5/Red channels is expressed for negative control (C-), the results of whole series are considered false. It is required to eliminate contamination.

ATTENTION! A single negative test result, especially if it is a sample from the upper respiratory tract, does not exclude infection. Lower respiratory tract sampling should be checked for SARS-CoV-2 coronavirus RNA, especially in cases of severe and progressive disease.

Negative results do not eliminate the possibility of SARS-CoV-2 infection and should not be used as the only reason for taking a decision about patient treatment. Negative results should go together with clinical observations and epidemical information.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual).

The use of shortened amplification program is only acceptable for CITO diagnostics.

11. SPECIFICATIONS

a. The analytical **specificity** of the **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** was assessed by bioinformatics 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, three genome sites were selected as targets to improve the reliability of diagnostics: the N and E genes sites specific to the SARS-CoV-2 coronavirus, as well as the conservative E-gene site common to the group of SARS-CoV-like coronaviruses (including SARS-CoV and SARS-CoV-2).

In the samples of human biological material with SARS-CoV-2 coronavirus RNA, the detecting amplifier should register an increase in fluorescence on the Fam/Green, Rox/Orange and Cy5/Red detection channels.

In the samples of human biological material free of SARS-CoV-2 coronavirus RNA and SARS-CoV-like coronaviruses RNA, the detecting amplifier should register an increase in fluorescence on the Hex/Yellow detection channel, the increase in fluorescence on the Fam/Green, Rox/Orange, and Cy5/Red channels should be absent.

In the samples of biological material free of SARS-CoV-2 coronavirus RNA, which contains SARS-CoV-like coronaviruses RNA (SARS coronavirus (various isolates); as well as Bat SARS-like coronavirus (various isolates); Bat SARS coronavirus (various isolates); SARS-like coronavirus (various isolates); SARS-related coronavirus (various isolates); Rhinolophus affinis coronavirus; Coronavirus BtRs-BetaCoV), the detecting amplifier should register an increase in fluorescence on the FAM/green detection channel, the increase in fluorescence on the Rox/orange and Cy5/red detection channels should be absent.

There are not non-specific positive results of amplification of RNA sample in the presence of Influenza A virus, Influenza B virus, Human coronavirus HKU-1, Human coronavirus NL-63, Human rhinovirus, DNA of *Mycoplasma pneumonia, Streptococcus pneumonia, Chlamydophila pneumoniae, Haemophilus influenza, 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 of the **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit** is **10 copies of NA** per amplification tube for package S; **6 copies of NA** per amplification tube for packages A, N. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

Sensitivity depends on the sampling and the final volume of the extracted RNA (elution volume).

Sensitivity of 10 copies for NA per amplification tube for package S; 6 copies of NA per amplification tube for packages A, N corresponds to the following values of the RNA concentration in the sample in case of using NA extraction kits produced by DNA Technology:

№	Detected virus	**PREP-NA, PREP-NA-S** DNA/RNA extraction kits (elution volume 50 µL)	**PREP-MB-NA DNA/RNA Extraction Kit** (elution volume 50 µL)
1	SARS-CoV-like coronaviruses	500 copies /mL sample	2000 copies /mL sample
2	SARS-CoV-2 coronavirus, E-gene	500 copies /mL sample	2000 copies /mL sample
3	SARS-CoV-2 coronavirus, N-gene	500 copies /mL sample	2000 copies /mL sample

c. Diagnostic characteristics

Number of samples (n) - 192;

Diagnostic sensitivity (95% CI) - 100% (95.6-100%);

Diagnostic specificity (95% CI) – 100% (96.7-100%).

ATTENTION! The claimed specifications are guaranteed when RNA extraction is performed with **PREP-NA DNA/RNA Extraction Kit** REF P-002/1EU, **PREP-NA-S DNA/RNA Extraction Kit** REF P-007-N/1EU and **PREP-MB-NA DNA/RNA Extraction Kit** REF P-114-A/8EU, REF P-114-N/4EU.

12. TROUBLESHOOTING

Table 11. 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	-	PCR inhibition RNA extraction violation	Repeat RNA extraction Repeat whole test Resample

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

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

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

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our official representative in EU by quality issues of the **SARS-CoV-2/SARS-CoV Multiplex 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,

Russia, 117587, 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

https://www.dna-technology.com

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

Annex A

Shortened method for the DNA/RNA extraction from the tested material (nasopharynx and oropharynx smears) using PREP-NA DNA/RNA Extraction Kit

ATTENTION! The lysis buffer can form the precipitate. Dissolve it at 65 °C for 10 minutes prior to use.

- Mark the required number of 1.5 mL tubes according to the number of samples to be analyzed and negative control ("C-").
- Vortex the tube with RNA-IC "A" (from **SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit)**. Add 10 µL of the RNA-IC "A" into the each tube.
- Add 300 µL of the lysis buffer into the each tube avoiding contact of the pipette tip with an edge of the tube.
- Add 100 µL of the sample into the marked tubes. Add 100 µL negative control into the tube marked "C-".
- Close the tubes tightly. Vortex the tubes for 3-5 seconds.
- Incubate the tubes for 5 minutes at 65 °C.
- Vortex the tubes for 3-5 seconds.
- Add 400 µl of the precipitation buffer. Close the tubes tightly and vortex them for 3–5 seconds.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 10 minutes at room temperature (18-25 °C).
- Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.
- Add 500 µL of the wash solution №1 to the precipitate, close tubes and mix by inverting the tube 3-5 times.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 1 minute at room temperature (18-25 °C).
- Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.
- Add 300 µL of the wash solution №2 to the precipitate, close tubes and mix by inverting the tube 3-5 times.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 1 minute at room temperature (18-25 °C).
- Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample. It is allowed to leave the precipitate covering with liquid in the volume of no more than 20-30 µL.
- Open the tubes and dry the precipitate at 65 °C for 5 minutes.
- Add 50 µL of the dilution buffer to the precipitate. Vortex the tubes for 3-5 seconds and spin down the drops by centrifuging for 3-5 seconds.

ATTENTION! Dilution buffer differs for **PREP-NA and PREP-NA PLUS** DNA/RNA extraction kits. Using of dilution buffer from another kit is not allowed. Only Dilution buffer from **PREP-NA DNA/RNA Extraction** Kit can be used.

- Incubate the tubes for 5 minutes at 65 °C. Vortex the tubes for 3-5 seconds.
- Centrifuge the tubes at RCF(g) 12000 - 16000 for 30 seconds at room temperature (18-25 °C).
- The NA preparation is ready for RT-PCR.

ATTENTION! The resulting RNA preparation must be used immediately for RT-PCR. 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 defrost before reverse transcription.

R3-P436-23/9EU,
R3-P436-S3/9EU
R3-P436-XA/XEU
R3-P436-VA/XEU
R3-P436-N3/9EU

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