INSTRUCTION FOR USE

NAME

Detection kit for the SARS-CoV-2 RNA presence in biological material using real-time isothermal amplification method in different versions according to TU 21.10.60-004-06931260-2020
Version I. 24 Detections

Detection kit for the SARS-CoV-2 RNA presence in biological material using real-time isothermal amplification method in different versions according to TU 21.10.60-004-06931260-2020

The Kit includes:

1. Reagent E, transparent colorless liquid, 160 µl, 1 tube
2. Reagent P, transparent colorless liquid, 110 µl, 1 tube
3. Positive control sample, transparent colorless liquid, 150 µl, 1 tube
4. Negative control sample, transparent colorless liquid, 150 µl, 1 tube
5. Operational documents:

5.1 Datasheet
5.2Instruction for Use

MANUFACTURER

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INTENDED USE

Detection kit for the SARS-CoV-2 RNA presence in biological material using real-time isothermal amplification method in different versions according to TU 21.10.60-004-06931260-2020 (the "Kit") is intended for high-quality detection of SARS-CoV-2 RNA in nasopharyngeal/oropharyngeal smears and sputum taken from the patient.

The Kit is intended for clinical laboratory diagnostics.

INDICATIONS AND CONTRAINDICATIONS

The Kit is used in clinical laboratory diagnostics to analyze biological material taken from individuals with clinical symptoms of a respiratory disease or suspected of being infected with SARS-CoV-2, persons arriving from areas heavily affected by the epidemic, and persons who came in contact with them. The Kit can also be used for laboratory diagnostics of biological samples from persons who do not show signs of respiratory diseases and who were not in contact with SARS-CoV-2 infected persons, including the ability to gather such samples at airports or other transportation hubs.

There are no contraindications, except for cases when biological samples cannot be gathered for medical reasons. Do not use the
Kit if the inner packaging is damaged or the appearance of a reagent is not as described below.

Do not use the Kit, if transportation and storage conditions were not observed.

Do not use the expired Kit.

The Kit can be used for early diagnosis of the SARS-CoV-2 and for epidemiological monitoring.

PRODUCT SPECIFICATIONS

Kit components are disposable. The Kit does not require maintenance and calibration.

No.	Item	Quantity, pc
1	Detection kit for the SARS-CoV-2 RNA presence in biological material using real-time isothermal amplification method in different versions according to TU 21.10.60-004-06931260-2020	
1.1	Reagent E, transparent colorless liquid, 160 µl	1
1.2	Reagent P, transparent colorless liquid, 110 µl	1
1.3	Positive control sample, transparent colorless liquid, 150 µl	1
1.4	Negative control sample, transparent colorless liquid, 150 µl	1
1.5	Operational documents	---
1.5.1	Datasheet	1
1.5.2	Instruction for Use	1

160 µl of Reagent E is packed into a microtube of 1.2 ml volume. Composition: Trisma, Potassium Acetate, Ammonium Sulphate, Magnesium Sulphate, Tween 20, dNTP, Nuclease- free Distilled Water, AacPoll DNA Polymerase, AMV-RT Revertase.

110 µl of Reagent E is packed into a microtube of 1.2 ml volume. Composition: Nuclease-Free Distilled Water, 6 SmartAmp Asymmetric Primers.

150 µl of the positive control sample is packaged into a microtube of 1.2 ml volume. Composition: aqueous suspension of the *in vitro* stabilized RNA fragment of the synthesized SARS-CoV-2 coronavirus genome.

150 µl of the negative control sample is packaged into a microtube of 1.2 ml volume. Composition: DNase, RNase free Distilled Water. The Kit is packaged in
a foil/paper bag.

THE NUMBER OF ANALYZED SAMPLES

The Kit is designed for 24 detections including controls.

METHOD: The method is based on the detection of SARS-CoV-2 coronavirus RNA in biological material by real-time SmartAmp fluorescence-detecting isothermal amplification. The method uses the technology of nucleic acids isothermal amplification at the reaction temperature of 67 ℃.

METHOD LIMITATIONS: Virus RNA may not be detected in the infected material because the titer (concentration) of the virus is less than $1x10^4$ copies/ml.

Virus RNA may not be detected in infected material due to insufficient nucleic acid extraction efficiency. To achieve indicators of analytical sensitivity, the used extraction kit should ensure the efficiency of the NA extraction at a level of at least 20%.

The reason for obtaining a false-positive result is the reaction inhibition, the insufficient efficiency of the NA allocation. False positive result can be checked with the control samples included in the Kit.

PRODUCT ANALYTICAL AND DIAGNOSTIC SPECIFICATIONS

Limit of Detection (Analytical Sensitivity)

The analytical sensitivity for the analyzed biological samples is $1x10^4$ copies of SARS-CoV-2 coronavirus RNA per ml.

Analytical Specificity

The Kit does not cross-react when testing the RNA of Influenza A and B viruses, parainfluenza, adenovirus infection, respiratory syncytial infection, metapneumovirus infection, rhinovirus infection and human coronavirus infection caused by hCov NL63, hCov OC43, hCov 229E, hCov DNA HKI, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Staphylococcus aureus*.

Diagnostic Sensitivity

Diagnostic sensitivity is 100% (99.2% - 100%) with a confidence interval of 95%

INTERFERING SUBSTANCES AND RESTRICTIONS ON THE SAMPLE USE

In general, the following endogenous and exogenous substances can inhibit RNA extraction reducing the effectiveness of the reaction:

Endogenous factors: hemoglobin during the reaction reduces the output when the concentration in the reaction mixture is> 1 mg / ml, urea (in the urine ~ 350 mm) begins to inhibit at> 20 mm avg.

Exogenous factors: heparin (inhibits the reaction at a concentration of more than 0.15 U/ml).

Other information on the interfering substances associated with the test samples, subject to the collection and preliminary preparation of the samples, is not available.

Raw sputum samples are unsuitable for the test and thus should be processed by diluting with saline prior to performing the reaction.

REAGENT CONSUMPTION:

Name	Function	Required volume per reaction, µl
Reagent E	Target NA amplification enzymatic catalysis	6
Reagent P	Primers required to initiate the real-time isothermal amplification process and detect the fluorescence signal	4
Positive control sample	Positive control sample	10
Negative control sample	Negative control sample	10

TEST CONTROL:

Each Kit contains positive and negative control samples to control the reaction. The PCR reaction for coronavirus RNA and control samples detection is performed simultaneously.

The time required to achieve stable test results is no more than 30 minutes.

SAFETY MEASURES TO PROTECT THE OPERATOR

When using the Kit, comply with relevant guidelines. All components of the Kit, in the concentrations used, are non-toxic, they do not have a harmful effect on the operator's health. When working with the Kit, the usual laboratory precautions should be followed:

- Use personal protection equipment.
- Do not eat or drink, smoke at the workplace.
- Wash hands thoroughly after handling samples and reagents.

Avoid contact with skin, eyes and mucous membranes; rinse with plenty of water if components of the Kit get on them. If you accidentally ingest the components of the Kit, immediately seek medical help.

POSSIBLE EFFECTS OF ELECTROMAGNETIC FIELDS

When using the Kit, it is not required to take precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure drops, reloading, and sources of thermal ignition.

PRECAUTIONS TO BE TAKEN AGAINST SPECIAL RISKS

When using the Kit, there is no need to take precautions against any special risks during use and sale, since the products do not include substances of human or animal origin, taking into account their potential infectious nature.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Real-time amplifiers, for example CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). Use a Laminar Flow Cabinet equipped with a germicidal UV lamp.

EQUIPMENT USED

- A set of electronic or mechanical variable volume microliter pipettes, with a variable volume of (20-200 µl, 100-1000 µl)
- Laminar Flow Cabinet
- Microtube racks
- Microcentrifuge for Eppendorf-type tubes
- Vortex
- Refrigerator with temperature from +2 to + 8 °C, equipped with a freezer with temperature of up to -20 °C
- Disposal containers for tips and microtubes
- Disposable tips for variable volume with pipettes with aerosol barriers up to 200, 1000 µl (for example, Axygen, USA).
- Disposable polypropylene tightly closed microtubes with a volume of 0.2 ml or 0.5 ml (for example, Axygen, USA)

MATERIALS AND REAGENTS NOT INCLUDED INTO THE KIT:

- Individual disposable lab coats, masks, gloves.
- Disinfectant container.
- Sampling swabs.

PLEASE NOTE! When working with RNA, it is required to use only disposable sterile plastic consumables with a special RNase-free marking.

BIOLOGICAL MATERIAL COLLECTION, TRANSPORTATION AND STORAGE:

The sampling procedure should be carried out in accordance with epidemiological rules.

The collection of clinical material and its packaging is carried out by an employee of a medical organization trained in biological safety when collecting material suspicious of infection with microorganisms of the second pathogenicity group.

Transportation of the material to the laboratory is carried out at temperature of 2 to 8 °C.

The test samples are stored at a temperature of 2 to 8 °C for no more than 2 days, at a temperature of minus 20 to 16 °C for no more than one month, at a temperature of minus 70 °C or in liquid nitrogen for 1 year, preventing thawing to conducting a study.

When freezing clinical material, its transportation should also be carried out in a frozen state. Avoid repeated freezing and thawing of the samples.

PREPARATION OF REAGENTS FOR ANALYSIS AND TESTING

Sample - 0.01 ml of an RNA solution extracted from biological samples.

I. Sample Preparation:

1) Dispense 6 µl from the Reagent E tube into each test tube or each well of a 48- or 96-well PCR plate.
2) Mix 10 µl of the isolated RNA sample with 4 µl from the Reagent R tube, mix in a vortex.
3) Precipitate received 14 µl of the mixed sample in a microcentrifuge to remove droplets from the walls of the tube.
4) Add the mixed sample (14 µl) to each test tube or well of a 48- or 96-well PCR plate containing Reagent E.

II. Positive Control Sample Preparation:

5) Dispense 6 µl from the Reagent E tube into each test tube or each well of a 48- or 96-well PCR plate.
6) Use the Positive Control Sample tube as a positive control sample when filling test tubes or wells in a 48 or 96-well PCR plate. Mix 10 µl from the Positive Control tube with 4 µl of the Reagent P tube.
7) Add the mixed sample (14 µl) to each test tube or well of a 48- or 96-well PCR plate containing Reagent E.

III.Negative Control Sample Preparation:

8) Dispense 6 µl from the Reagent E tube into each test tube or each well of a 48- or 96-well PCR plate;
9) Use the Negative Control Sample tube as a negative control sample when filling test tubes or wells in a 48- or 96-well PCR plate. Mix 10 µl from the Negative Control Sample tube with 4 µl of the Reagent P tube;
10) Add the mixed sample (14 µl) to each test tube or well of a 48 or 96-well PCR plate containing Reagent E

AMLPIFICATION

1) Fill test tubes or a 48- or 96-well PCR plate according to the settings of the PCR amplifier used, seal with a film (if necessary) and load it into the PCR amplifier.
2) Perform an incubation reaction at 67 ℃, a cycle time of 60 seconds, a reaction time of at least 30 minutes. Use FAM channel for fluorescence measurement.

TEST RESULTS EVALUATION

Real-time PCR and registration of the results is carried out in accordance with the operational documents of the device used. Interpretation of the results and the reliability of the reaction is carried out by positive and negative controls.

The results are interpreted based on the presence (or absence) of the intersection of the fluorescence curve with the liminal line set at the appropriate level (which corresponds to the presence (or absence) of the liminal cycle value "Ct" in the corresponding column in the results table).

The results are subject to recording in case of:

a) there is an intersection of the fluorescence curves with a threshold line on the FAM channel in samples with positive control samples;
b) there is no positive signal on the FAM channel in the sample with negative control samples.

The result is considered positive if the fluorescence accumulation curve for the corresponding sample has a characteristic "sigmoid" shape and crosses the liminal line. The Ct value should be less than 30. The reaction time at the time of output should not exceed 30 minutes. The amplitude of the signal does not matter.

The result is considered negative if there is no Ct value on the FAM channel.

SHELF LIFE. TRANSPORTATION AND STORAGE CONDITIONS

Transportation. The Kit is transported by all types of covered transport in accordance with the rules of cargo transportation applicable to such a type of transport, at temperatures from -80 °C to -20 °C and relative humidity from 10 to 90% under conditions excluding the effect of aggressive environments, direct sunlight and moisture. Repeated freezing of the Kit is not allowed.

During transportation, loading and unloading of products, measures must be taken to protect the container from mechanical damage, exposure to atmospheric precipitation and aggressive environments.

Storage. The Kit in manufacturer's packaging must be stored at temperatures from -80 °C to -20 °C and relative humidity from 10 to 90% under conditions excluding the effect of aggressive environments, direct sunlight and moisture. Repeated freezing of the Kit is not allowed.

MARKING SYMBOLS

	Produced by		Caution! Refer to the instructions for use.
	Production date		Refer to the instructions for use
	Best before		Do not use if packaging is broken
	Batch number		Storage temperature range
	Research use only		Moisture protection
 **24**	The contents are designed for 24 tests		Biological hazard