Instruction for Use

NAME

Reagent kit for the SARS-CoV-2 RNA nucleic acids extraction from biological material in different versions according to TU 21.10.60-002-06931260-2020

Version I. 24 detections:

1.1. SSB solution, transparent colorless liquid, 25 ml, 1 tube
1.2. WS1 solution, transparent colorless liquid, 25 ml, 1 tube
1.3. WS2 solution, transparent colorless liquid, 25 ml, 3 tubes
1.4. EB solution, transparent colorless liquid, 3 ml, 1 tube
1.5. Filter column with eluate collection tube, 24 pcs
1.6. Operational documents:
1.6.1 Instruction for Use
1.6.2 Datasheet.

Version II. 96 detections:

2.1. SSB solution, transparent colorless liquid, 50 ml, 2 tubes
2.2. WS1 solution, transparent colorless liquid, 50 ml, 2 tubes
2.3. WS2 solution, transparent colorless liquid, 50 ml, 6 tubes
2.4. EB solution, transparent colorless liquid, 5 ml, 2 tubes
2.5. Operational documents:
2.5.1 Instruction for Use
2.5.2 Datasheet.

MANUFACTURER

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INTENDED USE

Reagent kit for the SARS-CoV-2 RNA nucleic acids extraction from biological material in different versions according to TU 21.10.60-002-06931260-2020 (the “Kit”) is intended for extraction of the SARS-CoV-2 RNA from nasopharyngeal and oropharyngeal swabs.
The Kit is intended for clinical laboratory diagnostics.
The Kit is used with Pretreatment device for the nucleic acids extraction from the biological samples for SARS-CoV-2 isothermal amplification performance according to TU 26.60.12-001-06931260-2020, Registration Certificate No. РЗН (RZN) 2020/10089 of 17 April 2020 (the “Device”).

Scope:

The Kit is used to extract the SARS-CoV-2 RNA from biological material swab specimens collected from people with clinical symptoms of a respiratory disease and people who came in contact with those infected with SARS-CoV-2, irrespective of their age, and from people of all ages without clinical symptoms of a respiratory disease (in heavily affected areas / regions with high infection transmission rate) to ensure early diagnosis of COVID-19 and prevent the spread of the infection.
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 met.
Do not use an expired Kit.
The Kit can be used for early diagnosis of SARS-CoV-2 and for epidemiological monitoring.
The Kit can be used by medical and healthcare institutions, virology laboratories and epidemiological services.
Potential users: Only medical personnel trained in molecular diagnostics and handling clinical diagnostic laboratory equipment in accordance with the established guidelines and rules are allowed to work with the Kit.

PRODUCT SPECIFICATIONS

The Kit components are disposable.
The Kit does not require maintenance and calibration.
The list of the Kit components for 24/96 detections is shown in the table below:

Pos.	Name	Quantity, pcs
1.	Version I. 24 detections:	---
1.1.	SSB solution, transparent colorless liquid, 25 ml	1
1.2.	WS1 solution, transparent colorless liquid, 25 ml	1
1.3.	WS2 solution, transparent colorless liquid, 25 ml	3
1.4.	EB solution, transparent colorless liquid, 3 ml	1
1.5.	Filter column with eluate collection tube	24
1.6.	Operational documents	---
1.6.1	Instruction for Use	1
1.6.2	Datasheet	1
2	Version II. 96 detections:	---
2.1.	SSB solution, transparent colorless liquid, 50 ml	2
2.2.	WS1 solution, transparent colorless liquid, 50 ml	2
2.3.	WS2 solution, transparent colorless liquid, 50 ml	6
2.4.	EB solution, transparent colorless liquid, 5 ml	2
2.5.	Operational documents:	---
2.5.1	Instruction for Use	1
2.5.2	Datasheet	1

SSB solution is a transparent colorless liquid, which comes in 25ml or 50ml (according to the version) plastic tubes with screw caps.
WS1 solution is a transparent colorless liquid, which comes in 25ml or 50ml (according to the version) plastic tubes with screw caps.
WS2, solution is a transparent colorless liquid, which comes in 25ml or 50ml (according to the version) plastic tubes with screw caps.
EB solution is a transparent colorless liquid, which comes in 3ml or 5ml ml (according to the version) plastic tubes with screw caps.

Contents:

Name of Kit component	Component	Final concentration in the reagent
SSB solution	Guanidine thiocyanate	2.4 mol
	Sodium polyacrylate	0.0025%
	Buffer Tris-HCl, pH 7.4	25 mmol
	Triton X-100	0.5%
	Ethanol	33%
WS1 solution	Guanidine hydrochloride	2.4 mol
	Ethanol	50%
WS2 solution	Ethanol	95%
EB solution	Nuclease-free distilled water	100%

Filter columns with eluate collection tubes, in the amount according to the version, packed in plastic zip-lock bags, are packed together with reagents in a foil bag.

Number of detections
The Kit is designed for 24/96 detections (according to the version).

Method
The extraction method is based on the selective adsorption of nucleic acids to the column filter material.
The extraction of the SARS-CoV-2 RNA from nasopharyngeal and oropharyngeal swab specimens using the Kit and Device is performed in 5 consecutive stages and consists of lysing a sample of biological material in SSB buffer solution, collecting the RNA on the filter column filter, clarifying and collecting eluate containing purified target RNA. The sample is then used for isothermal amplification.
The stages of the RNA extraction using the Device are the following:
-During the first stage of sample preparation, the biomaterial is suspended in the SSB lysis buffer. The lysis buffer is composed of ethyl alcohol and a chaotropic agent (guanidine thiocyanate) that give the buffer its protein denaturation properties and allow effective viral particle lyses. As a result, proteins composing viral capsid lose their structure, viral particle is disintegrated and viral genetic material is released into solution. COVID-19 genetic material is a single-stranded RNA that includes highly conserved sequence targeted for the detection.
-During the second stage, solution containing SSB lysis buffer together with lysed viral particles is passed through a filter column. The viral RNA is retained in the filter, while remaining biomaterial, together with SSB lysis buffer that would interfere with amplification reaction, are passed through the filter.
-Two successive washing steps are implemented in order to ensure complete removal of all interfering substances, the washing buffer WS1, and WS2 containing ethanol.
-During the next step, the filter membrane has to be dried completely to ensure no ethanol residue remains. Ethyl alcohol may interfere with isothermal amplification reaction by inhibiting nucleic acid amplification. During both washing and drying stages, the target RNA is retained within the filter membrane.
-During the final stage, RNase free ultra-pure water is passed through the filter to elute the target RNA that is accumulated in a collection tube.

Interfering substances:
Substances in concentrations as shown in the table below do not affect the efficiency of RNA extraction from biological samples:

Interferent	Concentration in a sample
Mucin	5% v/v

Reagent consumption:

Reagent	**Function**	**Required volume per sample**
SSB solution	Lysis buffer	1.0 ml
WS1 solution	Washing solution	1.0 ml
WS2 solution	Washing solution	1.0 ml
EB solution	Eluting solution	100 µl

SAFETY MEASURES FOR USING THE KIT
Laboratories performing tests for the SARS-CoV-2 RNA detection are required to ensure operational safety in accordance with the relevant guidelines.
During operation, keep to the following rules:
- Treat biological samples being tested as infectious hazard.
- When removing tubes containing PCR products, do not open or spray the contents as it can lead to PCR contamination of the laboratory room, equipment and reagents.
- Use the Kit strictly as intended and in compliance with this Instruction for Use.
- Only specially trained personnel are allowed to use the Kit.
- Do not use an expired Kit.
It is also necessary to ensure compliance by the staff with biosafety rules and perform necessary operations to prevent nucleic acid contamination of the samples being tested as well as the rooms and equipment.
The potential risk for using the Kit is rated as Class 3 (Order of the Russian Ministry of Healthcare No.4 of 6 June 2012).

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 (gloves and lab coats).
- Do not eat or drink, smoke at the workplace.
- Wash hands thoroughly with water and soap 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 FOR RNA EXTRACTION
- Operations with the Kit must be performed in desktop biological safety cabinets, e.g. Laminar Flow Cabinet.
The Kit is used with Pretreatment device for the nucleic acids extraction from the biological samples for SARS-CoV-2 isothermal amplification performance according to TU 26.60.12-001-06931260-2020.

Equipment used
- Pretreatment device for the nucleic acids extraction from the biological samples for SARS-CoV-2 isothermal amplification performance according to TU 26.60.12-001-06931260-2020, Registration Certificate No. P3H (RZN) 2020/10089 of 17 April 2020
- A set of electronic or mechanical variable volume microliter pipettes, with a variable volume of (20-200 µl, 100-1000 µl)
- Laminar Flow Cabinet of class II type A of biosafety.
- Micro-tube racks.

Glassware and labware
- Solid waste containers for used pipette tips and tubes.
- Disposable pipette tips for automatic pipettes with aerosol barrier of up to 200, 1000 µl (for example, Axygen, USA).
- Disposable polypropylene screw-cap 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 and gloves.
- Disinfectant container.
- Sampling swabs.

For Version II, 96 detections:
- Filter columns (spin columns) of nominal volume of 1.2ml with filters (registered as required by law).
- Eluate collection tubes of nominal volume of 1.2ml (registered as required by law).

Measuring equipment and consumables for sampling used should be registered as medical devices.
CAUTION! When working with RNA, it is required to use only disposable sterile plastic consumables with a special RNase-free marking.

SAMPLE DESCRIPTION
Sample type
Clinical nasopharyngeal and oropharyngeal swab specimens are used as material for RNA extraction.
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 should be 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.
Sample collection timing is very important, since the highest level of virus content in the human respiratory tract may be during the first 4 days after the early signs of the disease. Samples should be collected during the first 3 days after the initial onset of the disease.
Each sample must be packed into an individual container.
Do not freeze samples after thawing.
All samples collected for laboratory testing should be considered potentially infectious, and medical personnel who collect or transport clinical samples must strictly comply with biological safety rules.
Healthcare staff who collect samples must use appropriate personal protective equipment (PPE).
Immediately after taking the sample, the tube should be marked, indicating the full name and age of the patient, the day of illness, type of material, date and time of sampling. Other details of the patient, i.e. the estimated diagnosis, date of admission, clinical symptoms, the patient's medical history (including therapy, epidemiological data including foreign trips within 21 days before the onset of signs of the disease, risk factors), etc. are provided in the supporting documentation.
Sample transportation and storage:
Samples are transported to the laboratory at 2°C to 8°C within a day according to relevant regulations.
Samples are stored at 2°C to 8°C for no more than 2 days, at -20°C to -16°C for no more than one month and at -70°C or in liquid nitrogen for one year. No thawing is allowed prior to testing.
If samples have been frozen, transportation must be carried out in the frozen state. Only one freezing and thawing cycle is allowed.
Safety measures for handling samples.
Potentially infected biological material (samples) must be recorded, stored and transported in strict compliance with relevant guidelines and regulations.
Samples must be disposed of in accordance with local guidelines.

REAGENT PREPARATION AND EXTRACTION
- The RNA extraction is performed using Pretreatment device for the nucleic acids extraction from the biological samples for SARS-CoV-2 isothermal amplification performance according to TU 26.60.12-001-06931260-2020, Registration Certificate No. P3H (RZN) 2020/10089 of 17 April 2020

1. Preparation:
I. Install the tray into the Device.
II. Firmly fit Rack 1 onto the tray.
III. Firmly install 8 empty DNA LoBind, PCR-clean tubes included in the Reagent Kit in the holes of Rack 3 for collecting the eluate.
IV. Firmly fit Rack 2 onto Rack 1.
V. Firmly install 8 filter columns included in the Reagent Kit in the holes of Rack 2.

NOTE! Do not close the lids of the filter columns!
2. Sample preparation:
I. Perform sampling with a swab. Products used to collect material must be certified as medical products.
II. Pipette 1 ml of the SSB reagent into a DNA LoBind, PCR-clean Eppendorf disposable tube of 2 ml volume to suspend the patient sample.
III. Place the swab into a tube containing 1 ml of the SSB solution. Rotate the swab in the tube for ~20 seconds, squeezing the swab against walls of the tube. Dispose of the swab according to the local clinical waste disposal regulations. The resulting lysate does not contain any viable viruses (it is achieved by the composition of the SSB solution) and can be used in further pretreatment procedure.

3. Sample extraction:
I. Carefully pipette 500 µl of the SSB solution containing your sample into a filter column and press the Control button. Closely monitor filtration and once all the solution has passed through the silica membrane filter, press the Control button again to turn the device's vacuum system off.
Repeat this step with the remaining 500 µl of the sample.
II. Carefully pipette 500 µl of the WS1 reagent into the filter column and press the Control button. Closely monitor the WS1 reagent to ensure it has completely passed through the silica membrane filter in the column. Once complete, press the Control button again to turn the device's vacuum system off.
Repeat this step 1 more time to the total of 1ml of WS1 solution used.
III. Pipette 700 µl of the WS2 reagent into the filter column and press the Control button. Closely monitor the WS2 reagent filtration to ensure solution has completely passed through the silica membrane filter in the column. Once complete press the Control button to turn the device's vacuum system off.
Repeat this step 3 more times to the total of 2.8ml of WS2 solution used.
IV. Press the Control button to initiate the DRYING step. Perform DRYING for at least 5 minutes. Visually monitor the process to ensure complete silica membrane drying.
While performing the DRYING step, insert 2 ml collection tubes in Rack 3, if haven't done so yet.
V. Replace Rack 1 with Rack 3 containing the eluate collection tubes.
VI. Carefully pipette 120 µl of the EB reagent directly onto the silica membrane and press the Control button. After the EB reagent has completely passed through the membrane filter, press the Control button again to turn the device's vacuum system off.
VII. Make sure the liquid has accumulated at the bottom of the tube. Use the eluate (extracted RNA sample) in amplification.

SHELF-LIFE. TRANSPORTATION AND STORAGE

The shelf-life is 12 months[1].* Do not use an expired Kit.

The Kit is stable within 12 months[1]* after unpacking and until the expiry date marked on the Kit.

Note: To maintain the declared stability after opening the package, all manipulations with EB solution should be carried out under aseptic conditions preventing contamination of the solution.

After opening the package, the Kit must be stored under the storage conditions and test tubes with solutions must be closed tightly.

Transportation The Kit is transported by all types of covered transport in accordance with the rules of cargo transportation applicable to this type of transport, at +2°C to +30°C, under conditions excluding the effect of aggressive environments, direct sunlight and moisture. 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 +2°C to +30°C under conditions excluding the effect of aggressive environments, direct sunlight and moisture. Freezing of the Kit is not allowed.

Safe disposal

Used components, which have contacted biological samples, are subject to disinfection and then must be stored in waste collection containers or plastic bags and disposed of as Class C extremely hazardous waste. Kits that have become unusable, inclusive of expired Kits and Kits that were opened but not used, are disposed of as Class D waste. The packaging is disposed of as Class A waste. Waste classification might differ in your country, please contact the manufacturer if additional info is required.

Warranty

The manufacturer warrants the Kit functional specifications meet the requirements of specifications and operational documents within the established shelf-life (12 months), provided that the transportation and storage conditions are met.

Forward your claims to: EVOTECH-MIRAI GENOMICS Limited Liability Company
address: 3B Akademika Artsimovicha St, Room 11, Moscow 117437, Russia
e-mail: mail@evotech-mg.com

Any side effects not listed in this Instruction for Use, undesirable reactions, facts or conditions posing a threat to the health and life of people and medical staff and associated with the use of the Kit should be reported to the manufacturer, EVOTECH-MIRAI GENOMICS LLC, at the above address, as well as to the relevant governmental supervising authority as required by the applicable law.

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 damaged
	Batch number		Storage temperature range
	Research use only		Moisture protection
 24 / 96	The contents are designed for 24/96 tests		

[1]Note:* - Not verified by real time testing.