HANDBOOK
LifeCase COVID-19

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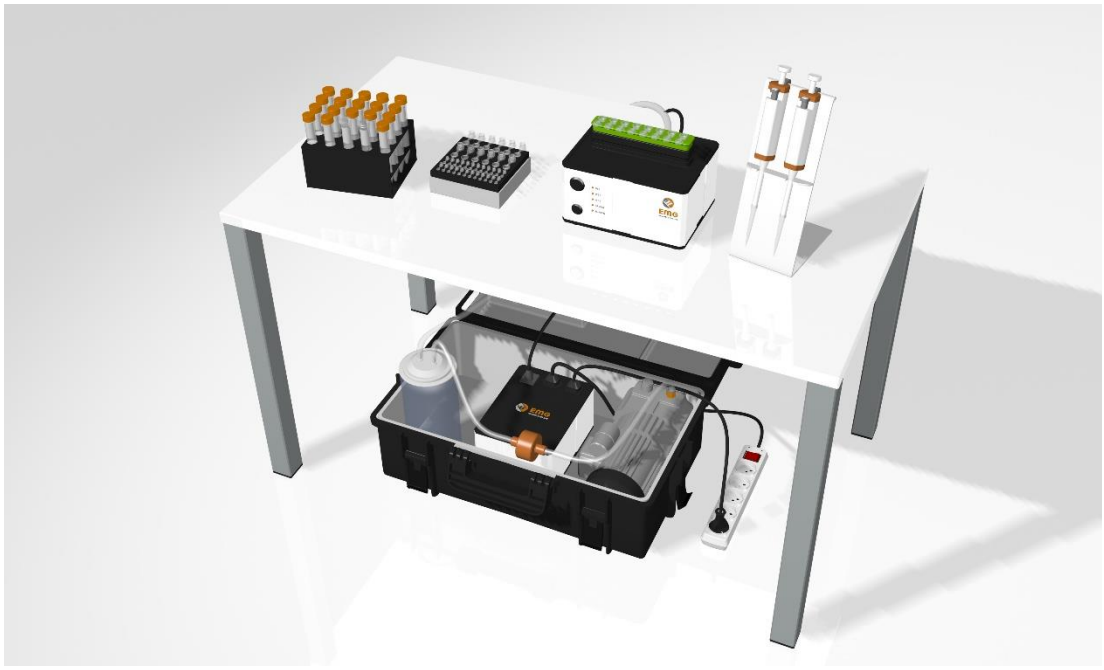
CASE 1 Pre-PCR



CASE 2 Post-PCR

Caution! The pre-PCR (RNA Extraction) and post-PCR (Amplification) handling zones should be in different rooms!

The portable mini laboratory "LifeCase COVID-19" includes:

Case 1

Pos.	Name: Pretreatment Device for the Nucleic Acids Extraction from the Biological Samples for SARS-CoV-2 Isothermal Amplification Performance including:	Quantity
1	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	1
2	Interface unit	1
3	Rack 1 (plugged unit)	3
4	Rack 2 (filtration unit)	3
5	Rack 3 (eluate collection unit)	3
6	Waste collection container with a lid	1
7	Bacterial air filter	2
8	Connection tube 1.8 m	1
9	Connection tube 0.6 m	1
10	Connection tube 0.45 m	1
11	Connecting wire	1
12	Compressor with connecting wire	1
13	Rubber O-ring	2
14	Power cord	1
15	Tube pressure gauge 0.15 m	1
16	Operational documents:	Enclosed inside Case 2
16.1	Handbook	1
16.2	Datasheet	1
17	Quick start guide	1

Additional equipment (optional)		
18	Automatic pipette 0.5 - 10 µl	1
19	Automatic pipette 100 - 1,000 µl	1
20	Microtube rack	1

Case 2

Pos.	Name: Isothermal Amplifier for SARS-Cov-2 Nucleic Acid Detection according to TU 26.51.53-003-06931260-2020 including:	Quantity
1	Isothermal amplifier for SARS-Cov-2 nucleic acid detection according to TU 26.51.53-003-06931260-2020 (the "Isothermal Amplifier")	1
2	Power supply unit	1
3	USB 2.0 High-speed A-B cable	1
4	USB drive with software	1
5	Tube rack, 2 ml	1
6	Straight tweezers	1
7	Power strip	1
8	Spacer without lenses	3
9	Operational documents:	
9.1	Handbook	1
9.2	Datasheet	1
10	Quick start guide	1
	Accessories:	
1	Laptop PC (optional)	1
2	Wireless mouse	1

Warnings and Precautions

When using the LifeCase, it is required to take precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure drops, restarting, and sources of thermal ignition.
Strong electromagnetic fields (generated by unshielded radio frequency sources) can interfere with proper operation and may lead to malfunction or incorrect results.
– Do not use the LifeCase near sources of strong electromagnetic fields, as these fields may interfere with proper operation.
– Assess the electromagnetic environment before operating the LifeCase.
– Reduce electromagnetic interference.

CAUTION!

Perform the extraction of the nucleic acids of viruses from biological samples using the Pretreatment Device and the preparation of the reaction mixture and the isothermal amplification of SARS-CoV-2 using the Isothermal Amplifier in different rooms.

Pretreatment Device

All sampling, transportation and laboratory testing operations must be carried out in strict compliance with your local applicable laws, standards and protocols. Coronavirus-related diagnostics, transportation, testing etc. might have special applicable guidelines, please consult your local authorities.

Isothermal Amplifier

Operations with viruses must be performed in a molecular biology (PCR) laboratory analyzing biological samples for the presence of disease-causing agents in compliance with your local applicable laws. There are special laws and guidelines that apply to handling, storing, registering, transporting and disposing of devices when working with materials containing pathogenic microorganisms.

During operation, keep to the following rules:

- Treat biological samples being tested as infectious hazard.
- Inactivate biological samples being analyzed in compliance with your local guidelines.
- Operation of these devices will produce waste that must be disposed of accordingly.

It is also necessary to ensure compliance by the staff with biosafety rules and guidelines and performing of such necessary operations to prevent nucleic acid contamination of the samples being tested as well as the rooms and equipment.

How to prepare a patient or a potentially infected person for swabbing

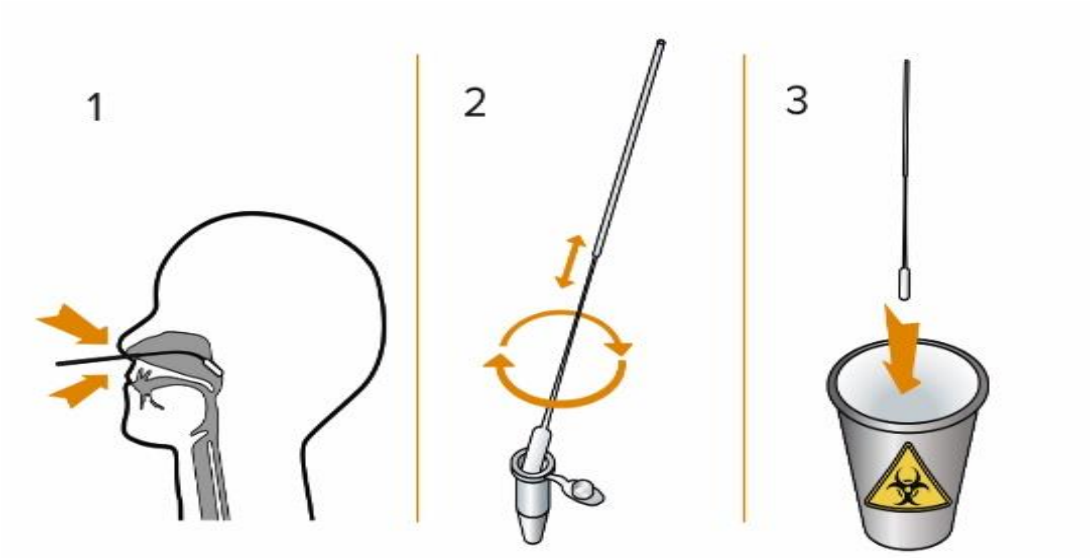
A patient or a potentially infected person should not drink or eat, gargle or wetten the throat at least 1 hour before swabbing. Nasal inhalers and use of oil-based sprays on the day of swabbing are not allowed. Spray use is allowed at least 1 hour before swabbing. The nose should be cleaned from excess secretions 1 hour before swabbing.

Swabbing procedure

Use the swab included into our "Reagent kit for the SARS-CoV-2 RNA nucleic acids extraction from biological material". To collect a biological sample, insert the nasopharyngeal swab into the nasal passage of a patient to such a depth where you feel a slight resistance to the further advancement of the swab; rotate the swab for 5 seconds.

Place the swab into a tube containing 1 ml of the SSB solution. Rotate the swab in the tube for ~15-20 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 further in the pretreatment procedure. It is recommended to immediately use the sample in the SSB reagent in further phases of the procedure. It is allowed to store the sample in the SSB reagent at room temperature for up to 12 hours.

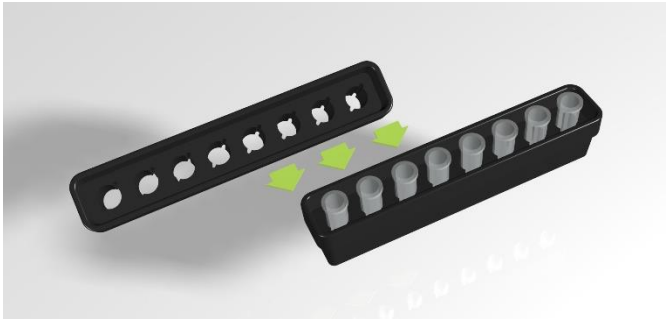


1. Preparation.

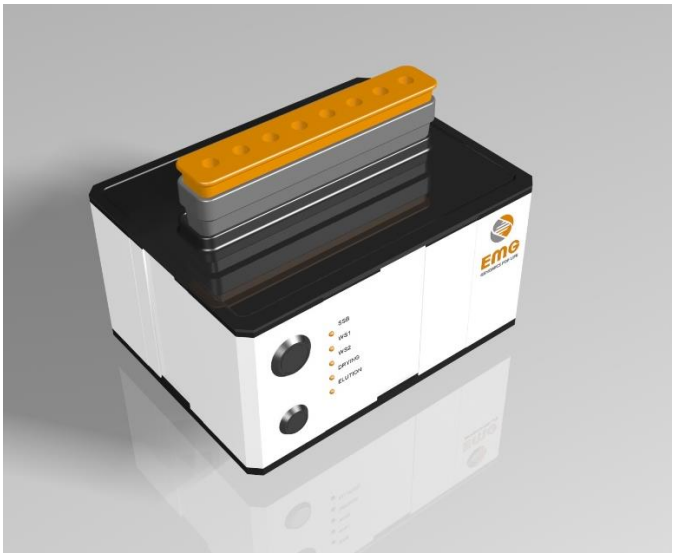
I. Firmly install the Tray (included into the Pretreatment Device set) into the respective slot of the Pretreatment Device.

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 the Rack 1.



V. Firmly install 8 filter columns included in the Reagent Kit in the holes of Rack 2.

Note: If less than eight (8) samples are to be used for RNA extraction, install empty filter columns and firmly close the lids of the columns where the samples are not to be pipetted.

Caution! While operating the Pretreatment Device, do not close the case where the compressor is placed to avoid overheating.

2. Sample Extraction.

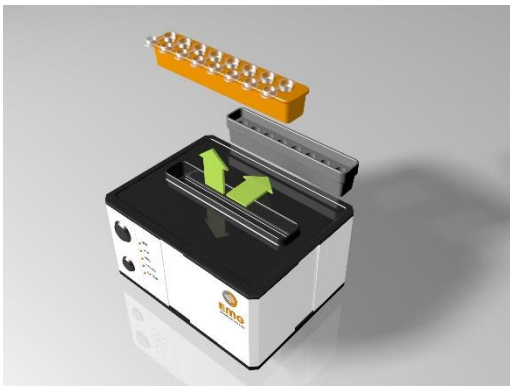
I. Carefully pipette 700 µl of the SSB solution containing your sample into each of the 8 filter columns installed in Rack 2. Press the Control button, it will turn the Pretreatment Device's vacuum system on, and a typical noise will be audible. Visually monitor that the SSB reagent with the sample has completely passed through the filter in all the columns. Press the Control button to turn the Pretreatment Device's vacuum system off.

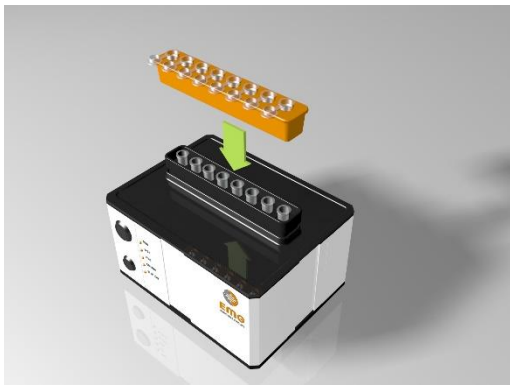
II. Without changing the position of Rack 2, carefully pipette 800 µl of the WS1 reagent into each of the 8 filter columns. Press the Control button, it will turn the Pretreatment Device's vacuum system on. Visually monitor that the WS1 reagent with the sample has completely passed through the filter in all the columns. Press the Control button to turn the Pretreatment Device's vacuum system off.

III. Without changing the position of Rack 2, pipette 800 µl of the WS2 reagent into each of the 8 filter columns. Press the Control button, it will turn the Pretreatment Device's vacuum system on, and a typical noise will be audible. Visually monitor that the WS2 reagent with the sample has completely passed through the filter in all columns. Press the Control button to turn the Pretreatment Device's vacuum system off. Repeat the stage one more time.

IV. Without changing the position of Rack 2, press the Control button, it will turn the Pretreatment Device's vacuum system on. Perform the DRYING phase for 5 minutes. Press the Control button, it will turn the Pretreatment Device's vacuum system off. Visually monitor that there is no sign of WS2 reagent on column tips.

V. Replace Rack 1 with Rack 3 containing the eluate collection tubes. Check the Racks and the eluate collection tubes for tightness.





VI. Sequentially pipette 100 µl of the EB reagent into 8 filter columns, right to the center of the filter, and press the Control button, it will turn the Pretreatment Device's vacuum system on. Visually monitor that the EB reagent has passed through the filter. Press the Control button, it will turn the Pretreatment Device's vacuum system off.

VII. Remove Rack 3 with the inserted Rack 2, make sure the liquid has accumulated at the bottom of the eluate collection tube.

VIII. Move the solution from eluate collection tubes into the PCR tubes. Immediately move to Section 4 of this Handbook.

IX. Disinfect all the Racks and the Tray by submerging into the disinfectant solution in accordance with your local applicable disinfection guidelines.

CAUTION: Immediately proceed to amplification mixture preparation.

Recommendations: It is highly recommended to run negative control along each round of the RNA isolation. Negative control is pure SSB solution with no viral biomaterial.

If sample components or used reagents are on the surface of the Pretreatment Device, disinfect the surfaces by wiping in compliance with effective in-house rules.

3. SARS-CoV-2 RNA Detection.

Have all the reagents completely thawed and then thoroughly mixed before use. Make sure there are no drops on the caps of the tubes.

Reagent P is light-sensitive, store in a dark place and avoid direct light.

Mark the tubes before placing them into the Isothermal Amplifier.

CAUTION: Reagent P is light-sensitive. Avoid exposure to light and store in a dark place.

I. Carefully pipette 4 µl of the Reagent P tube into each test tube.

II. Add 10 µl of the extracted RNA sample, mix by pipetting. Incubate the mixture for 4 minutes (but not longer than 5 minutes) at room temperature.

III. Add 6 µl of the Reagent E to the obtained 14 µl of the mixed sample and mix by pipetting.

Positive Control Sample Preparation:

I. Carefully pipette 4 µl from the Reagent P tube into the positive control sample tube.

II. Add 10 µl from the Positive Control Sample tube, mix by pipetting.

III. Add 6 µl from the Reagent E tube to the obtained 14 µl of the mixed sample, mix 5 times by pipetting.

Negative Control Sample Preparation:

I. Carefully pipette 4 µl from the Reagent P tube into the negative control sample tube.

II. Add 10 µl from the Negative Control Sample tube, mix by pipetting.

III. Add 6 µl from the Reagent E tube to the obtained 14 µl of the mixed sample, mix 5 times by pipetting.

Recommendations: To prevent contamination during RNA extraction, use the eluate solution produced by extraction from SSB without putting a sample into it as the negative control.

4. Amplification.

CAUTION: To have the optimum temperature to perform isothermal amplification, turn on the Isothermal Amplifier at least 15 minutes prior the amplification reaction. Avoid moving, shaking or causing any vibration to the Isothermal Amplifier during amplification.

I. Install the prepared test tubes into the wells of the Isothermal Amplifier for SARS-Cov-2 Nucleic Acid Detection.
II. Select the necessary block (A/B/C) and start the amplification by pressing the Start button in the Amplifier Client software.
III. Perform amplification rection at 65 ºC, with the cycle time of 60 seconds, expected reaction time of minimum 40 minutes

Note: If a real-time amplification device is used (e.g., CFX96 Touch, BioRad), set the following amplification settings: incubation at +67ºC, cycle time - 60 seconds, expected reaction time - minimum 40 minutes. Set the registration of the fluorescent signal to be performed at the end of each minute. Use FAM-type filters. Amplification and results recording are performed in accordance with the operational documents of the device.

Results Evaluation

Interpretation of the results and the reliability of the reaction is carried out by the positive and negative controls.
The results are interpreted based on the presence (or absence) of the rise of the fluorescence curve and the cycle threshold (Ct) value in minutes for the Isothermal Amplifier for the SARS-CoV-2 RNA detection.

Note: If a real-time amplification device is used (e.g., CFX96 Touch, BioRad), the results are interpreted based on the presence (or absence) of the intersection of the fluorescence curve with the threshold line set at the appropriate level, which corresponds to the presence (or absence) of the Ct cycle threshold value (in the relevant line of the result evaluation table for a real-time amplification device like CFX96 Touch, BioRad).

The results are subject to recording in case of:

a) the appearance of the fluorescence curve for the control sample
b) the absence of a positive signal on the channel of the negative control sample.

The result is considered positive if the fluorescence accumulation curve for the corresponding sample has a characteristic "sigmoid" shape and crosses the threshold. The Ct value must be lower than 30 (for CFX96 Touch Real-Time PCR Detection System). Reaction time (at the time of the result output) should not exceed 30 minutes. The result is considered negative if there is no rise in the fluorescent curve.
The test report can be exported as a PDF document by clicking "Export to PDF".

Note: A clear fluorescence curve crossing the threshold line after 30 minutes of the reaction may indicate inhibition of the reaction. In this case it is recommended to repeat the test.

Storage and transportation

The Reagent Kit for the SARS-CoV-2 RNA Nucleic Acids Extraction from Biological Material in Different Versions must be transported and 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.

The Detection Kit for the SARS-CoV-2 RNA Presence in Biological Material Using Real-Time Isothermal Amplification Method in Different Versions must be transported and stored at -80°C to -20°C under conditions avoiding effects of aggressive environment, direct sunlight and moisture. Only one freeze/thaw cycle is allowed. After thawing, the Kit can be only stored at +4°C for 5 days.

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.

For matters relating to the quality, please contact EVOTECH-MIRAI GENOMICS Limited Liability Company at 7 Universitetskaya St, Room 68, Innopolis city, Republic of Tatarstan, Russia 420500 Tel. 8-800-7076401

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