HANDBOOK
LifeCase COVID-19

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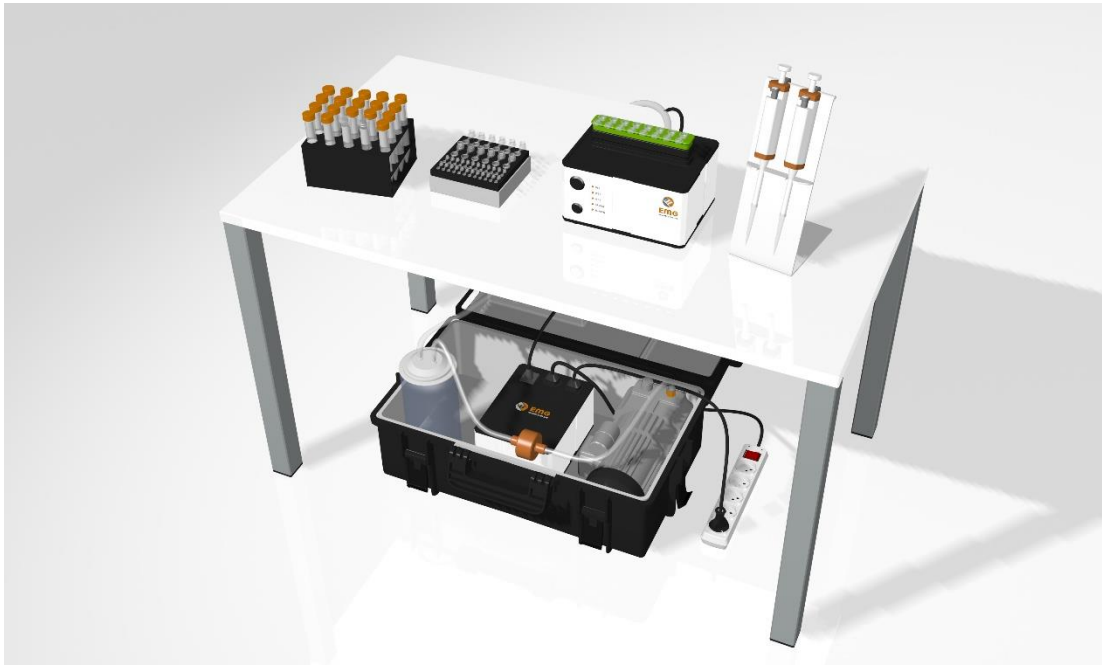
CASE 1 Pre-PCR



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	Tray	1
4	Rack 1 (plugged unit)	1
5	Rack 2 (filtration unit)	1
6	Rack 3 (eluate collection unit)	1
7	Waste collection container with a lid	1
8	Bacterial air filter	1
9	Connection tube 1.8 m	1
10	Connection tube 0.6 m	2
11	Connecting wire	1
12	Compressor	1
13	Compressor connecting wire	
14	Power cord	1
15	Operational documents:	Enclosed inside Case 2

15.1	Handbook	1
15.2	Datasheet	1
Additional equipment (optional)		
16	Automatic pipette 0.5 - 10 µl	1
17	Automatic pipette 100 - 1,000 µl	1
18	Microtube rack	3

Case 2

	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	Operational documents:	
5.1	Handbook	1
5.2	Datasheet	1
	Accessories:	
1	Laptop PC (optional)	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

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.

Dispose of the swab in accordance with your local guidelines.

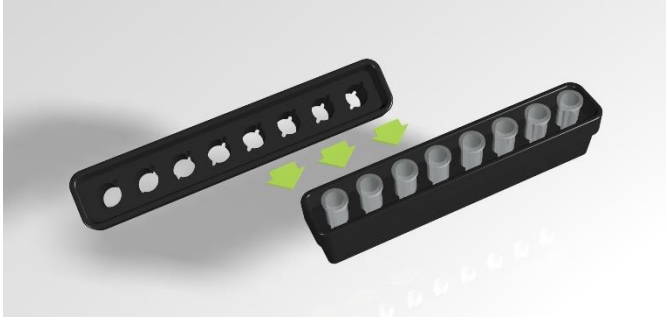
The resulting lysate does not contain any viable viruses (it is achieved by the composition of the SSB solution) and can be used further in the pretreatment procedure. It is recommended to immediately use the sample in the SSB reagent in further phases of the procedure. It is allowed to store the sample in the SSB reagent at room temperature for up to 12 hours.

1. Preparation:

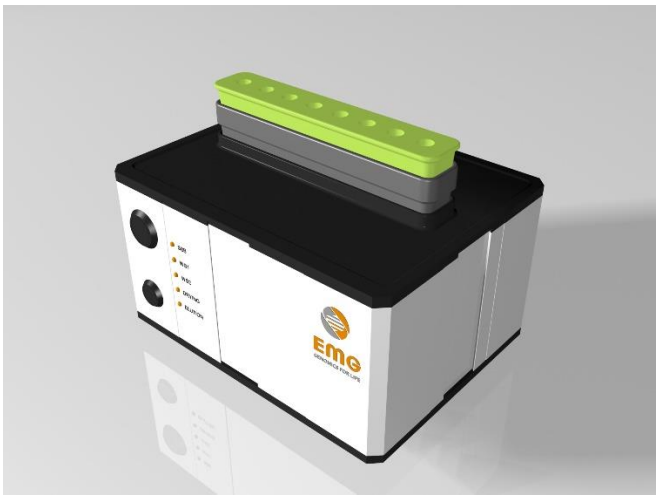
I. Firmly install the Tray (included into the Pretreatment Device set) into the respective slot of the Pretreatment Device.

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.

2. Sample Extraction:

I. Step 1: SSB

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. Step 2: WS1

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. Step 3: WS2

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. Step 4: DRYING

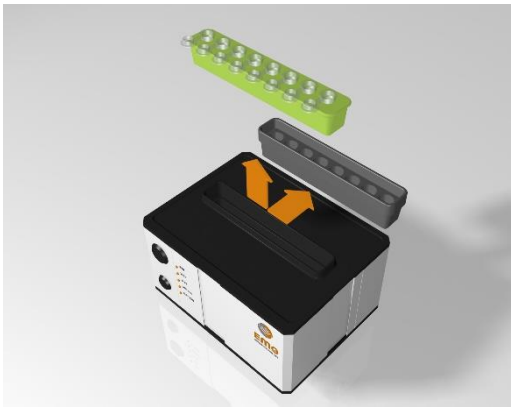
Press the Control button to initiate the DRYING step. Perform DRYING for at least 5 minutes. Visually monitor the process to ensure complete silica membrane drying.

While performing the DRYING step, insert 2 ml collection tubes in Rack 3, if haven't done so yet

Note: If WS2 reagent stays at the tips of the columns, this can lead to inhibition of the amplification reaction and, as a result, to a false negative result.

V. Step 5: ELUTION

Replace Rack 1 with Rack 3 containing the eluate collection tubes. Fix



VI. Step 6:

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. Step 7:

Visually check the liquid has accumulated in the eluate collection tube located in Rack 3. Collected solution is an eluate containing extracted RNA from the sample. The eluate solution can now be used in the amplification reaction.

VIII. Step 8:

After completing all stages of pre-treatment, disconnect Rack 2 from Rack 3. Transfer the eluate from Rack 3 to the corresponding signed tube and use it in accordance with the amplification instructions.

IX. Step 9:

Disinfect Racks with a 3% hydrogen peroxide solution adding 0.5% detergent or with 1% chloramine solution.

NOTE: Immediately proceed to preparing for isothermal amplification.

Recommendations: when performing the pretreatment procedure, use the Negative Control Sample for data interpretation reliability. For these purposes, all the sample extraction stages (SSB, WS1, WS2, Drying, Elution) must be performed on one of the columns, without placing the Sample into SSB.
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:

I. Pipette 4 µl of Reagent P into each 0.2 ml test tube.
II. Add 10 µl of the extracted RNA sample into each test tube, mix by pipetting.
III. Add 6 µl of Reagent E into each test tube, mix 5 times by pipetting.

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

Positive Control Sample Preparation:

I. Pipette 4 µl of Reagent P into the positive control tube.
II. Add 10 µl from the Positive Control Sample tube, mix by pipetting.
III. Add 6 µl of Reagent E, mix 5 times by pipetting.

Negative Control Sample Preparation:

I. Pipette 4 µl of Reagent P into the negative control tube.
II. Add 10 µl from the Negative Control Sample tube, mix by pipetting.
III. Add 6 µl of Reagent E, mix 5 times by pipetting.

Recommendations: When performing the pretreatment procedure, use the Negative Control Sample for data interpretation reliability. For these purposes, all the sample extraction stages (SSB, WS1, WS2, Drying, Elution) must be performed on one of the columns, without placing the Sample into SSB.

4. Amplification:

CAUTION: To have the optimum temperature to perform isothermal amplification, turn the Isothermal Amplifier on at least 15 minutes prior the amplification reaction.

I. Install the prepared test tubes into the slots of the Isothermal Amplifier for SARS-Cov-2 Nucleic Acid Detection.

II. Start the amplification by pressing the Start button in the Amplifier Client software.

Note: If a real-time amplification device is used (e.g., CFX96 Touch, BioRad), set the following amplification settings: incubation temperature - +67ºC, cycle time - 60 seconds, expected reaction time - minimum 40 minutes (40 cycles), but 35 cycles must be analyzed. 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. If the curve rises after 35th minute, please repeat the test.

CAUTION: Avoid moving, shaking or causing any vibration to the amplifier during amplification, this might affect your results.

Results Evaluation

Interpretation of the results and the reliability of the reaction is carried out by positive and negative control. 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 threshold cycle 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 threshold cycle 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 in 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 40 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. IMPORTANT: After thawing, the Kit can be only stored for 5 days at +2 to +5°C.

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 117437, Moscow, Ak. Artsimovich Str., 3B, of.11.