SARS-CoV-2-ELISA-AG
SARS-CoV-2 Nucleocapsid Protein Antigen Detection Kit

Instruction for Users

REF #KS092

CE

LabPack LLC, Address: Karpovka River Embankment, 5, letter I, pom. 3-N, Saint-Petersburg 197376, Russia, phone: +7(812) 490-75-93, e-mail: info@labpack.ru

IVD

EC REP MagnaLab LLC, Address: Ludwika Rydygiera Str. 8/4, Warsaw 01-793, Poland,
phone: +48 531 261 817, e-mail: info@magnalab.pl

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Table 1. Abbreviations

SARS-CoV-2-ELISA-AG	SARS-CoV-2-ELISA-AG SARS-CoV-2 Nucleocapsid Protein Antigen Detection Kit
ELISA	Enzyme-linked immunosorbent assay
MP	Microplate
OD	Optical density
a.u.	Absorbance units (optical density units)
SF	Seropositivity factor

1. INTENDED USE

SARS-CoV-2-ELISA-AG SARS-CoV-2 Nucleocapsid Protein Antigen Detection Kit is intended for the qualitative detection of SARS-CoV-2 nucleocapsid antigen by enzyme-linked immunosorbent assay (ELISA) in nasopharyngeal and oropharyngeal swabs.

For professional use in clinical laboratory diagnostics; for examination of male and female patients without age limitation with clinical symptoms of respiratory disease with suspected COVID-19 infection.

Demographic and population aspects of the application: SARS-CoV-2-ELISA-AG is recommended for examination of male and female patients without age limitation.

Target analyte: nucleocapsid (N) protein of SARS-CoV-2 coronavirus.

Research type: qualitative assay.

Sample type: nasopharyngeal swabs or combined nasopharyngeal and oropharyngeal swabs.

The functional purpose of the test performed using the SARS-CoV-2-ELISA-AG is examination of patients with suspected COVID-19 infection to determine the presence of the SARS-CoV-2 nucleocapsid antigen in nasopharyngeal and oropharyngeal swab.

Application: professional use in clinical laboratory diagnostics and research practice. For *in vitro* diagnostic only.

Users' qualification requirements: a specialist with a higher or secondary specialized medical education, trained in licensed laboratory diagnostics courses.

2. PRINCIPLES OF THE PROCEDURE

The working principle of the SARS-CoV-2-ELISA-AG is based on the enzyme-linked immunosorbent assay.

Monoclonal antibodies to the nucleocapsid protein of the SARS-CoV-2 virus are immobilized on the surface of the polystyrene plate. At the first stage, during the incubation of the test samples and control samples in the wells of the plate in the presence of monoclonal antibodies labeled with biotin, the antigen (if present in the sample) binds to antibodies with the formation of immune complexes. When the incubation is over, all unbound components are removed from the reaction zone during the washing process.

At the second stage, the streptavidin-horseradish peroxidase conjugate binds to the previously formed immune complexes. When the incubation is over, all unbound components are removed from the reaction zone during the washing process.

When a tetramethylbenzidine solution is added, the solution in the wells becomes colored. After stopping the reaction with a stop solution and measuring the optical density (OD) of the solution in the wells, the seropositivity factor is calculated. The degree of coloration is proportional to the amount of SARS-CoV-2 antigen in the test samples. The presence of antigen to SARS-CoV-2 is assessed relative to the Cut-off value.

Detection of SARS-CoV-2 nucleocapsid antigen in test samples proceeds in three stages:

1) Preparation of samples for analysis
2) Conduction of ELISA procedure
3) Interpretation of the results

3. KIT DESCRIPTION

3.1 SARS-CoV-2-ELISA-AG description

SARS-CoV-2-ELISA-AG is available in one complete set (**Table 2**), designed for 96 tests, including control samples. Optionally, it is possible to conduct 4 independent tests for small sets of clinical samples.

Table 2. SARS-CoV-2-ELISA-AG description

Component	Component Description	Specifications	Amount
MP	Microplate – a modular plate with immobilized monoclonal antibodies to the SARS-CoV-2 nucleocapsid protein	96 well plate, clear colorless wells	1 piece
PC	Positive Control Sample *(based on SARS-CoV-2 nucleocapsid recombinant antigen in a stabilizer)*	Red liquid	1 ml x 1 bottle
NC	Negative Control Sample *(based on the culture of human epithelial cells in a stabilizer)*	Green liquid	2 ml x 1 bottle
IB	Incubation Buffer	Transparent pink liquid	10 ml x 1 bottle
CS-1	Conjugate 1 Solution *(Biotin-labeled monoclonal antibody solution)*	Colorless transparent liquid	8 ml x 1 bottle
CC-2	Conjugate 2 Concentrate *(streptavidin-horseradish peroxidase conjugate)*	Yellow or orange liquid	0.3 ml x 1 bottle
CCD-2	Conjugate 2 Concentrate Diluent	Transparent liquid, yellow or orange	15 ml x 1 bottle
TMB	Tetramethylbenzidine Substrate Solution	Colorless transparent liquid	12 ml x 1 bottle
Wash Buffer (20 x)	20x Concentrated Wash Buffer *(phosphate buffered saline with Tween)*	Colorless transparent liquid	20 ml x 1 bottle
Stop-solution	0.5 M Sulfuric Acid	Colorless transparent liquid	12 ml x 1 bottle
Reagent Tray	Disposable reagent tray	Transparent smooth surface, without contamination or mechanical damage	3 pieces
Plate Cover	Disposable microplate sealing film	–	2 pieces
User manual			1 piece
Certificate of quality			1 piece

The kit contains all necessary components, except for purified water.

3.2 Control materials included in SARS-CoV-2-ELISA-AG

PC – Positive Control Sample of SARS-CoV-2-ELISA-AG is based on SARS-CoV-2 nucleocapsid recombinant antigen.

NC – Negative Control Sample of SARS-CoV-2-ELISA-AG is based on the culture of human epithelial cells. Used to assess the correctness of the assay during routine setting.

4. PERFORMANCE CHARACTERISTICS

4.1 Analytical specificity

The influence of potentially **interfering substances** on the operation of the reagent kit was tested in relation to potentially interfering substances that might occur during the procedure for collecting biological material:

1. Hemoglobin 150 g/l
2. Mucin 5% v/v
3. "Ibuprofen" 0.04 mg/ml
4. "Ambrobene" 0.003 mg/ml
5. "Bromhexine" 0,016 mg/ml
6. "Kaletra" 0.02 mg/ml
7. "Interferon" 0.2 U/ml
8. "Teraflu" 0.071 mg/ml

Upon the tests results potentially interfering substances, evaluated at concentrations that would be expected to occur during normal use of the reagent kit, do not interfere with the assay result.

4.2 Cross reactivity

Table 3. The pair of antibodies was tested for cross-reactions with the following pathogens:

Recombinant antigens	**Cross reactivity**
Influenza B (B/Florida/4/2006) Nucleoprotein (His Tag) 40438-V08B	–
Influenza A H1N1 (A/California/07/2009) Nucleoprotein (His Tag) 40205-V08B	–
Human coronavirus (HCoV-HKU1) Nucleoprotein (His Tag) 40642-V07E	–
Human coronavirus (HCoV-OC43) Nucleoprotein 40643-V07E	–
Human coronavirus (HCoV-229E) Nucleoprotein (His Tag) 40640-V07E	–
Human coronavirus (HCoV-NL63) Nucleoprotein (His Tag) 40641-V07E	–
Viral lysates:	
Influenza A (H7N9)	–
HCoV OC43	–
Parainfluenza Type1	–
Parainfluenza Type2	–
Parainfluenza Type3	–
Influenza A (H2N2)	–
HCoV E229	–
Influenza A (H1N1) pdm09 Guangdong-Maonan	–
Influenza A (H3N2) HongKong/2671/2019	–
Influenza A (H5N1)	–
Influenza B Washington 02/2019	–
Influenza B Phuket	–
Human respiratory syncytial virus	–
Adenovirus	–

4.3 Diagnostic specificity

The diagnostic specificity of SARS-CoV-2-ELISA-AG (with a confidence level of 95%) based on the results of examining 124 clinical samples (oropharyngeal/nasopharyngeal swabs) characterized as not containing SARS-CoV-2 RNA by PCR analysis was 99 (95% CI 95 % – 100%).

The specificity determined with the Internal Standard Panel "SARS-CoV-ELISA-AG-ISP", certified by the manufacturer in accordance with the documents regulating the quality, is 100%.

4.4 Diagnostic sensitivity

The diagnostic sensitivity of the reagent kit was determined basing on the results of examining 87 clinical samples (oropharyngeal/nasopharyngeal swabs) from 87 patients diagnosed with COVID-19 infection.

The diagnostic sensitivity was 98 % (95% CI 89% – 100%).

The sensitivity determined with the Internal Standard Panel "SARS-CoV-2-ELISA-AG-ISP", certified by the manufacturer in accordance with the documents regulating the quality, is 100%.

4.5 Limits of detection

The lower detection limit of SARS-CoV-2-ELISA-AG to the antigen was:

- 5 pg/ml (for PBS as transport medium);
- 25 pg/ml (for UTM® (COPAN Diagnostics Inc) as transport medium).

The high-dose hook effect of SARS-CoV-2-ELISA-AG was not detected in testing the recombinant SARS-CoV-2 nucleoprotein up to the concentration of 100 000 pg/ml.

4.6 Reproducibility

Reproducibility for SARS-CoV-2-ELISA-AG assay was evaluated for two positive internal standards (low and high levels). Three investigative sites assessed the device's repeatability, within one analysis, between different analysis and total reproducibility.

CV (coefficient of variation) of seropositivity factor for the positive sample within one analysis does not exceed 8%.

CV of seropositivity factor for the positive sample between different analyses does not exceed 8%.

Reproducibility – CV for two positive internal standards (low and high levels) does not exceed 15%.

5. CLINICAL MATERIAL RECOMMENDATION

The material for the assay is clinical samples, including nasopharyngeal and oropharyngeal swabs. For best results, mix nasopharyngeal and oropharyngeal swabs together.

According to WHO guidelines «Diagnostic testing for SARS-CoV-2» the optimal specimen depends on clinical presentation and time since symptom onset. Respiratory specimens:

• Upper respiratory specimens are adequate for testing early-stage infections, especially in asymptomatic or mild cases. Testing combined nasopharyngeal and oropharyngeal swabs from one individual has been shown to increase sensitivity for detection of respiratory viruses and improve the reliability of the result. Two individual swabs can be combined in one collection tube or a combined nasopharyngeal and oropharyngeal swab can be taken. A few studies have found that individual nasopharyngeal swabs yield a more reliable result than oropharyngeal swabs.

• Lower respiratory specimens are advised if collected later in the course of the COVID-19 disease or in patients with a negative URT sampling and there is a strong clinical suspicion of COVID-19. LRT specimens can consist of sputum, if spontaneously produced (induced sputum is not recommended as this poses an increased risk of aerosol transmission). Before implementing other respiratory or oral fluid sampling methods, the sampling method should first pass validation in the laboratory for the intended

patient groups.

Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation and in the laboratory is essential.

Specimen type	Collection materials	Recommended temperature for storage and/or shipment to the laboratory and until testing (from date of specimen collection) [#]
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs with VTM *	2–8 °C if ≤12 days* minus 70 °C (dry ice) if > 12 days
Sputum	Sterile container	2–8 °C if ≤ 2 days minus 70 °C (dry ice) if > 2 days

[#] Avoid repeated freezing and thawing of specimens. If no access to minus 70 °C, consider storing at minus 20 °C.

** For transport of specimens use PBS or transport medium UTM® (COPAN Diagnostics Inc)*

6. MATERIALS REQUIRED BUT NOT SUPPLIED*

The materials and equipment required to work with the SARS-CoV-2-ELISA-AG are listed in **Table 5**.

Table 5. Materials and Equipment

Equipment/Materials	Specifications	Remarks (example)
Laminar box	Class II of biological safety	BAVp-01-Laminar-S-1,2. Laminar systems, Russia
Manual or automatic 1-channel variable pipettors	Vol.: 5–50 µl; 20–200 µl, 100–1000 µl	Sartorius Biohit, Finland
Manual or automatic 8-channel variable pipettors	Vol.: 5–50 µl, 50–300 µl	Sartorius Biohit, Finland
Pipettor tips	For variable pipettors with Vol.: 5–50 µl; 20–200 µl, 100–1000 µl	Axygen, USA
Polypropylene tubes	Vol.: 1.5–2 ml	Axygen, USA
Volumetric cylinders	Class II accuracy, max Vol.: 100 ml; 1000 ml	
Shaker with temperature control (incubator)	Temperature +37±1 °C, speed up to 800 rpm	SIA BIOSAN, Latvia
Automatic plate washer	For immunological plates	BIO-TEK Instruments, Inc, USA
Spectrophotometer with vertical scanning	Microplate reader capable of absorbance measurement at 450 and at 620–700 nm	Tecan Austria GmbH, Austria
Refrigerator	With chambers that maintain a temperature from +2 to +8 °C and with a freezer that maintains a temperature not higher than minus 16 °C	
Biohazardous waste container with a lid	For class B medical waste	
Personal Protective Equipment	Disposable dressing gown, hat, mask, gloves	

** In the absence of the above equipment and materials, it is allowed to use other means with characteristics no worse than those given.*

7. INSTRUCTIONS FOR USE

Before use check the integrity of the primary packaging, check the expiration date of the product, recover all reagents at room temperature for 30 min.

7.1 Assay requirements

Failure to comply with the requirements described below may result in distortion of the assay results!

7.1.1. Do not use expired reagents.

7.1.2. When using SARS-CoV-2-ELISA-AG for conducting several independent series of analysis, it should be kept in mind that the number of independent experiments is limited by the volume of the components.

7.1.3. **Positive Control** and **Negative Control** samples are to be used in each assay. It is unacceptable to evaluate the results using the values measured for **PC** and **NC** in a different series of analysis.

7.1.4. Use disposable vessels and pipettor tips with **TMB** and the Conjugate solutions (**CS-1** and **CC-2**).

7.1.5. Do not allow drying the content of the microplate wells between the operations.

7.1.6. Do not reuse the **Reagent Tray**.

7.1.7. The **Plate Cover** (sealing film) is for single use only. Do not use the film once it is peeled off. When using a part of the kit, cut a piece of the film of the corresponding size for each stage of incubation.

7.1.8. Do not use components from the kits of different batches, as well as reagents from other manufacturers.

7.2 Preparation of the components for the assay

Recover all the components of the kit at room temperature for 30 min prior to use.

Kit component	*Preparation of the components*
Microplate (**MP**)	Place the required number of strips on the frame. Pack the remaining strips in a bag with a desiccant and close the fastener tightly.
Positive Control sample (**PC**)	Ready to use
Negative Control sample (**NC**)	Ready to use
Incubation Buffer (**IB**)	Ready to use
Conjugate 1 Solution (**CS-1**)	Ready to use
Conjugate 2 Concentrate (**CC-2**)	Ready to prepare **Diluted Conjugate 2 Solution** (**DCS-2**) for further use (see paragraph 7.4.1.)
Conjugate 2 Concentrate Diluent (**CCD-2**)	Ready to prepare **Diluted Conjugate 2 Solution** (**DCS-2**) for further use (see paragraph 7.4.1.)
Tetramethylbenzidine Substrate Solution (**TMB**)	Ready to use
Wash Buffer (20x)	Ready to prepare **Wash Buffer (1x)** (see paragraph 7.4.2.)
Stop-Solution	Ready to use

7.3 Preparation of test samples

7.3.1. Place the required number of polypropylene tubes in the rack. Add 50 µl of **IB** into each tube*.

7.3.2. Resuspend thoroughly the test samples (combined nasal and oropharyngeal swab; or nasopharyngeal swab) by pipetting or vortexing. Add 200 µl of the suspension to the tubes with **IB***. Shake thoroughly the resulting mixture.

7.3.3. Incubate the tubes at room temperature for 30 min.

with an insufficient amount of the test sample, it is acceptable to change the volumes of **IB and the sample while maintaining the **IB** to sample volume ratio (for example, 25 µl of **IB** and 100 µl of the sample)*

7.4 Preparation of the reagent solutions

7.4.1. Preparation of the **Diluted Conjugate 2 Solutions**.

ATTENTION! Conjugate 2 Concentrate (CC-2) is only used in diluted form for further operations. The **Diluted Conjugate 2 Solution (DCS-2)** is to be prepared just before use. **DCS-2** is stable for 30 min at room temperature.

To prepare **DCS-2**, add to the **Reagent Tray** the required amounts of **Conjugate 2 Concentrate Diluent** (**CCD-2**) and **Conjugate 2 Concentrate (CC-2)** with the volume ratio of 50:1 according to **Table 6**. Stir the resulting solution thoroughly.

Table 6. Amounts of reagents required to prepare the Diluted Conjugate 2 Solutions

	Number of strips used in the assay											
	1	2	3	4	5	6	7	8	9	10	11	12
Component	**Diluted Conjugate 2 Solutions**											
CC-2, µl	20	40	60	80	100	120	140	160	180	200	220	240
CCD-2, ml	1	2	3	4	5	6	7	8	9	10	11	12

7.4.2. Preparation of **Wash Buffer (1x)**.

Shake thoroughly the bottle with **Wash Buffer (20 x)**. Dilute the required amount of **Wash Buffer (20 x)** 20 times with purified water. For example, mix together 10 ml of **Wash Buffer (20 x)** with 190 ml of water. Stir the solution thoroughly avoiding foaming.

7.5 Assay procedure

7.5.1. Recover all the components of the kit at room temperature for at least 30 min prior to use.

7.5.2. Add 50µl of **Conjugate 1 Solution (CS-1)** (biotin-labeled monoclonal antibody solution) to each well of the Microplate (**MP**).

7.5.3. Add the control reagents as follows:

- add 100 µl of **IB** to one well of the **MP** ("conjugate control");
- add 100 µl of **PC** to one well of the **MP**;
- add **NC** to two wells of the **MP** – 100 µl to each well;
- add the test samples (prepared in accordance with paragraph 7.3.) to the remaining wells – 100 µl to each well.

ATTENTION! Duration of the operations listed in paragraphs 7.5.2 and 7.5.3 should not exceed 20 min! Do not allow drying the content of the **MP** wells between any of the operations!

7.5.4. Seal the used **MP** wells with the **Plate Cover** (cut a piece of the sufficient size if needed) and incubate the **MP** at +(37±1) °C and shaking speed of 600–800 rpm for 60 min.

7.5.5. At the end of the incubation time, remove the **Plate Cover** and discard the liquid from each well (use a biohazard container with a lid). Fill the wells with **Wash Buffer (1x)** prepared as instructed in paragraph 7.4.2 (300 µl per well), mix by tapping, incubate for at least 30 seconds and discard the liquid from the wells. Repeat washing 3 times. During the washing procedure, avoid overflowing the wells, avoid touching the wells with the pipettor tips. After the last wash, remove thoroughly the residual liquid from the wells by tapping the MP in upside down position over tissue paper.

If using an automatic washer, use the shake function for 5 seconds after filling the wells with **Wash Buffer (1x)**. Check that the wells are properly filled and that the liquid has been removed completely.

7.5.6. Add 100µl of the **Diluted Conjugate 2 Solutions** (**DCS-2**) (see paragraph 7.4.1) to each well of the **MP.**

ATTENTION! Prepare **DCS-2** just before use. **DCS-2** is stable for 30 min at room temperature.

7.5.7. Seal the used **MP** wells with the **Plate Cover** and incubate the **MP** at +(37±1) °C and shaking speed of 600–800 rpm for 15 min.

7.5.8. When the incubation time is over, peel off the **Plate Cover** and discard the liquid from each well (use biohazardous waste container with a lid). Wash the wells and remove the residual liquid as described in paragraph 7.5.5.

7.5.9. Add 100 µl of Tetramethylbenzidine Substrate Solution (**TMB**) to each well of the **MP**. Incubate the **MP** protected from light at +(37±1) °C for 15 min.

7.5.10. Stop the reaction by adding 100 µl of **Stop-solution** to each well of the **MP**.

ATTENTION! The time between stopping the reaction and registering the results should not exceed 10 min.

8. REGISTRATION OF RESULTS

The results of ELISA are to be registered with a spectrophotometer that is capable of optical density (OD) measurement at 450 nm (main wavelength) and at 620–700 nm (background measuring wavelength).

The results registration is only acceptable at 450 nm wavelength. The background level is measured in air.

The measurements are to be carried out in 2–3 min after stopping the reaction. The OD values should be collected not later than 10 min after stopping the reaction.

9. INTERPRETATION OF RESULTS

The assay results are considered reliable if all the following conditions are met:
-OD values in wells with **PC** are more than 0.800 a.u.;
-OD values in wells with **NC** are less than 0.200 a.u.;
-OD values in wells with **IB** ("conjugate control") are less than 0.200 a.u.

9.1. Interpretation of ELISA results

Using formula (1), calculate the average optical density of the negative control sample (**NC**):

$$OD(NC)_{av} = \frac{OD(NC)_1 + OD(NC)_2}{2} \quad (1)$$

where OD (**NC**)$_{av}$ – average value of OD in the well with negative control sample (**NC**) (a.u.)
OD (**NC**)$_{1, 2}$ – value of OD in each well with **NC** (a.u.)

Using formula (2), calculate the Cut-off value (C.O.):

$$C.O. = OD(NC)_{av} + \alpha \quad (2)$$

where C.O. – Cut-off value (a.u.);
α – coefficient determined with statistical processing method by the manufacturer, indicated in the quality certificate of the reagent kit batch.

Using formula (3), calculate seropositivity factor for each test sample (SF):

$$SF = \frac{OD_{sample}}{C.O.} \quad (3)$$

where SF – seropositivity factor;
OD_{sample} – optical density of the sample (a.u.);
OD_{crit} – critical optical density value (a.u.).
Interpretation of the obtained results is described in **Table 7**.

Table 7. Interpretation of the obtained results

SF < 0.9	Negative result: test sample does not contain nucleocapsid protein of SARS-CoV-2 virus
0.9 ≤ SF ≤ 1.1	Questionable result: samples with questionable results are recommended to be retested. If the repeated obtained result is uncertain, the sample should be tested again, after 1–2 days. In case of uncertain results, such samples are considered to be negative
SF > 1.1	Positive result: test sample contains nucleocapsid protein of SARS-CoV-2 virus

Samples with optical density above the detection limit of the spectrophotometer are considered to be positive without further investigation.

10. REAGENT STORAGE AND TRANSPORTATION

The shelf life of the SARS-CoV-2-ELISA-AG is 12 months*.

The shelf life and storage conditions of the opened components of the product and working solutions are presented in **Table 8**.

Table 8. Shelf life and storage conditions of the opened components and working solutions from the reagent kit SARS-CoV-2-ELISA-AG

№	Component/working solution	Conditions and shelf life
1	Microplate (MP)	Store in a sealed bag with a desiccant during the shelf life of the SARS-CoV-2-ELISA-AG reagent kit at temperatures from +2 to +8 °C
2	Positive control sample (PC)	Store in the manufacturer's packaging for 1 month at temperatures from +2 to +8 °C
3	Negative control sample (NC)	
4	Incubation buffer (IC)	
5	Conjugate 1 Solution (CS-1)	
6	Conjugate 2 Concentrate (CC-2)	
7	Conjugate 2 Concentrate Diluent (CCD-2)	
8	Tetramethylbenzidine Substrate Solution (TMB)	Store in the manufacturer's packaging during the shelf life of the SARS-CoV-2-ELISA-AG reagent kit at temperatures from +2 to +8 °C
9	20x Concentrated Wash Buffer	
10	0.5 M sulfuric acid solution (Stop reagent)	
11	1x Wash Buffer	Store closed at room temperature from +18 to +25 °C for not more than 5 days.
12	Diluted Conjugate Solution 2	Stable for 30 min at temperatures from +18 to +25 °C.

**Fast track stability data of a medical device, not supported by real-time studies.*

10.1 Transportation

SARS-CoV-2-ELISA-AG can be transported by covered transport (road, rail or air) in thermal containers with ice packs at temperatures from +2 to +8 °C. Transportation is allowed at temperatures up to +27 °C during 1 day.

Do not use the kits transported in violation of the temperature regime.

10.2 Storage conditions

Store SARS-CoV-2-ELISA-AG at temperatures from + 2 to + 8 °C. Do not use the kits stored in violation of the regulated regime.

11. WARNINGS AND SPECIAL PRECAUTIONS

- SARS-CoV-2-ELISA-AG is intended for single use for the number of samples indicated on the package.
- The reagent kit is intended for professional use only, not intended for self-testing. Only personnel trained in diagnostic methods and working rules in a clinical diagnostic laboratory in accordance with the established procedure are allowed to work with the reagent kit. Biological material (nasopharyngeal/oropharyngeal swabs) can be collected by the patient in compliance with the instruction.
- The work must be carried out in compliance with the sanitary and epidemiological rules EN 13641:2002, EN ISO 15190:2020, Directive 2008/98/EC, EN ISO 15190:2020.
- The test samples should be considered as infectious, the work and storage should be organized in accordance with EN 13641:2002.
- In the process of work, it is imperative to use personal protective equipment: disposable gloves, laboratory coats. All materials, tools and equipment used, the workplace should be disinfected in accordance with the requirements of EN 13641:2002 and EN ISO 15190:2020.
- When working with the kit, avoid contact of the kit components with the skin, eyes and mucous membranes. In case of contact, immediately rinse the affected area with water and seek medical attention.
- It is allowed to use the kit strictly for the intended purpose, in accordance with these instructions and within the indicated expiration date.
- Do not use the reagent kit if the inner packaging is broken or the appearance of the reagent does not correspond to the description.
- Do not use the reagent kit if the conditions of transportation and storage have not been observed according to the instructions.
- Do not use the components from kits of different batches or produced by other manufacturers.
- The reagent kit is not subject to installation, assembly, adjustment, calibration, sterilization, use in combination with other medical devices or other manipulations.
- The reagent kit is non-sterile; it does not emit hazardous or potentially hazardous radiation levels when used as directed; it does not contain drug products for medical use; it contains the negative control sample made on the basis of a culture of human epithelial cells.
- No side effects were found while using the reagent kit.

12. DISPOSAL OF THE REAGENT KIT

- Unused reagents, expired reagents, as well as used reagents, packaging, biological material, including materials, tools and items contaminated with biological material, are to be discarded in accordance with the requirements of Directive 2008/98/EC in EU or relevant regulations in the consumer's country.
- Wastes containing potentially containing pathogens (materials, tools and items contaminated with biological fluids) are attributed to class B and must be decontaminated / neutralized. The choice of the method of disinfection / neutralization is determined by the capabilities of the organization carrying out medical activities. In the absence of a disinfection / neutralization site or a centralized medical waste disposal system, the kits are to be disinfected by the personnel by chemical / physical methods.
- When organizing areas for disinfection / neutralization of medical waste using hardware methods, it is acceptable to collect, temporarily store, transport class B medical waste without preliminary disinfection in places of generation, provided that the necessary epidemiological safety requirements are observed. The organization carrying out medical activities has to be provided with all the necessary consumables, including disposable packaging. All packaging has to be discarded.
- Disposal of the product in outer packaging with hazard warning signs must be carried out in accordance with appropriate safety and decontamination measures.

➢ The manufacturer, suppliers, sellers, importers can destroy kits that have lost their consumer properties or have expired, in accordance with Directive 2008/98/EC as class H9 medical waste using methods agreed with the territorial authorities responsible for sanitary and epidemiological well-being of the population. The personnel carrying out the destruction of the kits must comply with the safety rules for carrying out one or another method of destruction.

➢ All the components of the SARS-CoV-2-ELISA-AG, except for the Stop-solution component, are non-toxic. Sulfuric acid, which is a part of the specified component, has an irritating effect. In case of contact with skin and mucous membranes, rinse thoroughly the affected area with running water.

➢ IB, CS-1, CCD-2 contain ProClin 300 as a preservative, which can be absorbed through the skin and is an irritating agent, but is non-toxic in the concentrations used. In case of contact with skin or eyes, rinse immediately the exposed areas with water.

13. LIMITATIONS

Even though ELISA is a highly specific and highly sensitive method, false positive and false negative results are possible in rare cases since none of the known testing methods can give a complete guarantee of a positive and negative test result.

The reason for obtaining incorrect results may be non-compliance with the requirements and procedures described in this manual, including:

- violation of the integrity of the packaging during transportation,
- use of a kit with an expired shelf life,
- violation of the storage conditions of the kit;
- violation of the storage and transportation conditions of samples.

A negative result indicates that the test sample contains no detectable amount of the antigen. Samples with a SF in the range of 0.8–1.2 may contain a critically small value of the analyte and are subject to observation over time.

The diagnosis of an infectious disease should not be established based on the results of a single test using SARS-CoV-2-ELISA-AG, it should be determined by a competent specialist in combination with the results of other laboratory studies, the patient's history, the results of physical and instrumental studies.

14. MANUFACTURER'S WARRANTIES

14.1. SARS-CoV-2-ELISA-AG is manufactured by LabPack LLC. Address: Karpovka River Embarkment, 5, letter I, room 3-N, 197376 Saint-Petersburg, Russia.
Phone: +7 (812) 490 75 93.

14.2. The manufacturer guarantees the compliance of SARS-CoV-2-ELISA-AG with the requirements of the technical documentation if the conditions of transportation, storage and use established by this instruction for use are observed.

14.3. Instruction manual is provided by the manufacturer on paper and in the form of an electronic document via online publishing. The latest revision of the instruction manual: 04/09/2021.

14.4. Complaints about the quality of SARS-CoV-2-ELISA-AG are to be sent to an authorized representative of the manufacturer in the European Community:

MagnaLab LLC, Address: Ludwika Rydygiera Str. 8/4, Warsaw 01-793, Poland.

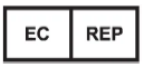
Phone: +48 531 261 817, e-mail: info@magnalab.pl

Annex A. Short protocol of assay procedure

No	Stage	Description	Temperature, duration	Remarks
1	Preparation of components	Recover all the components at room temperature	+18…+25 ºC for at least 30 min	–
2	Preparation of samples	Add 50 µl of **IB** into each tube. Add 200 µl of the sample suspension to the tubes. Shake thoroughly	+18…+25 ºC for 30 min	–
3	Preparation of wash buffer	Preparation of **Wash Buffer (1x)** (paragraph 7.4.2.)	+18…+25 ºC	–
4	Adding **CS-1**	Add 50µl of **CS-1** to each well of the **MP**	+18…+25 ºC	Operation time should not exceed 20 min
5	Adding **PC**, **NC**, "conjugate control", samples	- add 100 µl of **IB** to one well ("conjugate control"); - add 100 µl of **PC** to one well; - add **NC** to two wells – 100 µl to each well; - add the prepared test samples to the remaining wells – 100 µl to each well.	+18…+25 ºC	
6	Incubation	Seal the wells with **Plate Cover** and place the **MP** to a shaker with temperature control	+(37±1) ºC and shaking speed of 600–800 rpm for 60 min	–
7	Preparation **DCS-2**	Preparation of **Diluted Conjugate Solutions 2** (paragraph 7.4.1)	+18…+25 ºC	Prepare **DCS-2** just before use. **DCS-2** is stable for 30 min at room temperature
8	Washing	Wash each well 3 times with 300 µl of **Wash Buffer 1x**. Allow the buffer to contact with the content of the wells for 30 s each time	+18…+25 ºC	Check the correctness of the well filling and the completeness of the liquid removal if an automatic plate washer is used
9	Adding **DCS-2**	Add 100 µL of **DCS-2** to each well of the **MP**	+18…+25 ºC	–
10	Incubation	Seal the wells with **Plate Cover** and place the **MP** to a shaker with temperature control	+(37±1) ºC and shaking speed of 600-800 rpm for 15 min	–
11	Washing	Wash each well 3 times with 300 µl of **Wash Buffer 1x**. Allow the buffer to contact with the content of the wells for 30 s each time	+18…+25 ºC	Check the correctness of the well filling and the completeness of the liquid removal if an automatic plate washer is used
12	Adding **TMB**	Add 100 µL of **TMB** to each well of the **MP**	+18…+25 ºC	–
13	Incubation	Place the **MP** to an incubator or shaker with temperature control	+(37±1) ºC for 15 min	Protect the **MP** from light
14	Adding **Stop-solution**	Add 100 µL of **Stop-solution** to each well of the **MP**	+18…+25 ºC	–
15	Registration of results	Measure OD for each well at 450 nm. Use 620–700 nm wavelength to measure the background level	The OD values should be collected not later than 10 min after	–

			stopping the reaction	

Annex B. Symbols to be used with medical device labels

Symbol	Title of symbol	Symbol	Title of symbol
EN ISO 15223-1:2016			
	Use-by date		Do not re-use
	Date of manufacture		Consult instructions for use
	Batch code		Caution
	Catalogue number		*In vitro* diagnostic medical device
	Keep away from sunlight		Do not use if package is damaged
	Keep dry		Contains sufficient for <n> tests
	Temperature limit		Manufacturer
	Authorized representative in the European Community		
Regulation (EC) No 1272/2008			
	H290: may be corrosive to metals		
	H315: causes skin irritation		

Annex C. Harmonized standards

1	EN ISO 13485:2016	Medical devices – Quality management systems – Requirements for regulatory purposes
2	EN 13612:2002	Performance evaluation of in vitro diagnostic medical devices
3	EN 13641:2002	Elimination or reduction of risk of infection related to in vitro diagnostic reagents
4	EN 13975:2003	Sampling procedures used for acceptance testing of in vitro diagnostic medical devices – Statistical aspects
5	EN ISO 14971:2012	Medical devices – Application of risk management to medical devices
6	EN ISO 15223-1:2016	Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied – Part 1: General requirements
7	EN ISO 18113-1:2011	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions and general requirements
8	EN ISO 18113-2:2011	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 2: In vitro diagnostic reagents for professional use
9	EN ISO 23640:2015	In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents
10	EN ISO 62366-1-2015	Medical devices – Part 1: Application of usability engineering to medical devices
11	Directive 98/79/EC	Directive 98/79/EC of the European Parliament and of the Council on in vitro diagnostic medical devices
12	Directive 2008/98/EC	Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives
13	Regulation (EC) No 1272/2008	Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

The above standards were valid at the time the instructions for use were approved. In the future, when using the document, it is advisable to check the validity of the reference normative documents at the current moment. If the referenced document is replaced or changed, then when applying this document, the replaced (modified) document should be used.