SARS-CoV-2 S IgG ELISA
Instructions for use

Kit for in vitro diagnostic* IVD *use

Catalogue No.	Specific antibodies	Ig class	Substrate	Format
EI-2020-9499 G	SARS-coronavirus-2 (**SARS-CoV-2 S**)	IgG	Antigen-coated microplate wells	96 x 1 (96 wells)

1. Purpose of use

SARS-CoV-2 S IgG ELISA semi-quantitative immunosorbent assay (ELISA) test is used to determine the presence of human IgG class SARS-CoV-2 antibodies in blood serum or plasma with EDTA. These tests are used to confirm COVID-19 or an immune response to a pathogen. This test is useful in detecting possible viral infection outbreaks and their scale, helping to assess the proportion of the population exposed to the pathogen – epidemiological data. This product is designed for diagnostics IVD.

Note. *The test does not directly establish the presence of the virus. It identifies the antibodies that are specific for the virus or a vaccine, providing information on the immune response to the virus or a vaccine.*

2. Clinical significance of serological test

In order to control the spread of the SARS-CoV-2 virus and to prepare for its recurrence, it is essential to know, if an immune response to the virus has developed. Serological tests, which detect virus-specific antibodies, provide information about the immune system's response to a coronavirus infection or vaccine. In case of SARS-CoV-2, such testing is an additional diagnostic measure and recommended for establishing a former contact with the virus in patients with typical COVID-19 symptoms, and those, who show no symptoms (asymptomatic form).

The dynamics of the development of SARS-CoV-2 virus has not been sufficiently researched so far, but it is known that the majoritySARS-CoV-2 patients show an IgM and IgG antibody response at the same time or 10-21 days following the infection [1 2 3 4 5 6]. The IgM seroconversion median is established 12 days after the emergence of the symptoms, while IgG – after 14 [2]. About 65 days after the onset of the symptoms, the IgM level begins to drop until it reaches standard levels. Meanwhile, IgG level increases together with the IgM, but remains high for several months and longer[7]. In 79 % cases SARS-CoV-2 virus-neutralising antibodies are detected after 13-20 days, 98 % – after 28–41 days following the onset of the symptoms, correlating with detectable titre of S protein-specific antibodies [8]. Therefore, establishing the presence of SARS-CoV-2 antibodies is also important for the assessment of the protective immunity.

3. Antigen

ELISA microplate wells are coated with recombinant SARS-CoV-2 surface spike (S) glycoprotein, which has no transmembrane.

4. Test principle

During the first stage diluted patient blood samples are incubated in ELISA microplate wells with immobilised recombinant viral protein (antigen) - SARS-CoV-2 surface glycoprotein S. In case of a positive sample, virus-specific antibodies (IgG) will bind with the antigens. Bounded specific IgG

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antibodies are determined using enzyme-labelled antibodies (HRP, horseradish peroxidase) against the human IgG (enzyme conjugate). Horseradish peroxidase catalyses the colour reaction.

Depending on the amount of the antibodies in the samples, the solutions of different intensities are evaluated spectrophotometrically - by measuring the optical density.

5. Package content 96 x 1 (96)

Components	***Cap colour***	***Amount***
Antigen-coated ELISA microplate, ready to use	–	1 (96 wells)
Dilution buffer, ready to use	Brown	1 × 120 ml
Wash buffer, 10x concentrated	White	1 × 100 ml
Negative control (NC), ready to use	Blue	1 × 400 µl
Positive control (PC), ready to use	Red	1 × 300 µl
Enzyme conjugate, 120x concentrated	Green	1 × 200 µl
Enzyme substrate solution, 3,3',5,5'– tetramethylbenzidine solution, ready to use	Brown	1 × 12 ml
STOP solution, 3.6 % sulphuric acid solution, ready to use	Colourless	1 × 6 ml
Protective foil	–	3 pcs.
Instructions for use	–	1 pcs.
Quality control certificate	–	1 pcs.

6. Additional materials and equipment (not included into the package)

- Automatic ELISA microplate washing device (recommended). The microplates can also be washed manually with adherence to work safety requirements.
- ELISA microplate reader (parameters: 450 and 620 nm wavelength).
- Calibrated pipettes.
- Pipette tips.
- Dosing pipette: recommended for enzyme conjugate, enzyme substrate and STOP solution.
- Distilled or deionised water.
- ELISA microplate incubator – for incubation at +37°.
- The incubator or a water bath is recommended for heating the wash buffer.
- Watch or a stopwatch.

7. Storage and stability

The package contents must be stored at temperature between +2°C and +8 °C. **Important!** Do not freeze. As long as the packaged is sealed, all test reagents remain stable until the date of expiry, indicated on the package.

After opening, when stored at +2 to +8 °C and tightly closed, they remain stable until the date of expiry, indicated on the package, unless indicated otherwise in the instructions below.

8. Warnings and safety precautions

- The product must be used only by trained clinical research laboratory staff.
- If the packaged reagents are visibly damaged, do not use the kit.
- Read the instructions for use carefully before use. Use only the instructions, supplied together with the product in the same package.
- When administering the testing, follow the volumes, incubation time, temperature and preparation stage, indicated in the instructions.
- Do not change or mix IMUNODIAGNOSTIKA reagents with reagents of other manufacturers.
- Observe Good Laboratory Practice (GLP) and work safety requirements. Some of the reagents contain undeclared amounts of preservatives. Avoid the sample and reagent contact with eyes and skin. In case of contact with eyes or skin, wash thoroughly with water. Change or wash contaminated clothes. In case of swallowing, immediately seek for medical help.
- Positive and negative control are biological samples of human origin. Appropriate safety measures are recommended for working with these controls, the same as with blood samples.

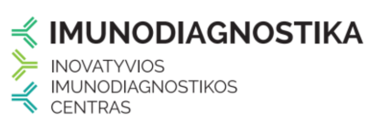
9. Sample preparation and stability

Human serum or plasma with EDTA must be diluted with the dilution buffer at the ratio of 1:101. For example, 10 µl sample must be diluted with 1 ml of dilution solution.
The samples can be stored at +2 to +8 °C for up to 14 days. It is recommended to dilute and use the samples on the day of conducting the testing.

10. Reagent preparation and stability

Note: all reagents must be taken out and kept at room temperature (+20°C to +25 °C) for 30 min.
Set the incubator for the microplate incubation at +37 °C.

- **ELISA microplate, coated with antigen.** Ready for use. Before opening the protective package of the microplate, the microplate must be kept at room temperature for 30 min. (Thus preventing the accumulation of moisture (condensate)).
- **Controls.** Ready for use. Mix the reagents well before use.
- **Enzyme conjugate.** Enzyme conjugate is a 120x concentrate. The enzyme conjugate must be diluted with a dilution buffer by adding 100 µl of the enzyme conjugate into 12 ml of the dilution buffer. **Note:** the enzyme conjugate must be prepared before use (step 2 of the instruction for use)
- **Dilution buffer.** Ready for use.
- **Wash buffer.** The wash solution is a 10x concentrate. In case of visually visible crystallisation in the concentrated wash buffer, heat it to +37 °C and mix well before dilution. The concentrate must be diluted with distilled water (dH_2O) at 1:10 (for example by adding 100 ml of wash buffer concentrate into 900 ml of dH_2O). **Note:** Ready for use wash solution remains stable storing at +2 to +8 °C for 4 weeks.
- **Enzyme substrate solution.** Ready for use. The bottle must be closed right after use – the reagent is light-sensitive. Note: blue substrate is unsuitable for use.
- **STOP solution.** Ready for use.



11. Waste management

Patient samples, controls and ELISA microplates must be managed as possible sources of infection. All reagents must be managed and disposed of in accordance with local laboratory waste treatment regulations.

12. Quality control

For every group of tests performed, the average optical density (OD) of the PC, NC and BC values obtained must match the parameters, provided for the specific package series. The package includes a quality control certificate with indicated quality control parameters. If test controls fail to match the parameters, the test result must be regarded as inaccurate and the test should be repeated.

13. Assay procedure

1 Step 1

13.1. Incubation with tested sample (serum or plasma)

Add **100 µl** of positive, negative and blank controls (dilution buffer) and prepared diluted patient samples (see pt. 9) into ELISA microplate wells. Cover the prepared microplate with a protective foil and incubate at +37 °C for 1 hour.

To increase the reliability of the results it is recommended to conduct two tests of each sample. The exemplary microplate filling scheme for testing 45 samples by conducting two tests for one patient is provided in the table.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC	S2	S6									S42
B	NC	S2	S6									S42
C	NC	S3	...									S43
D	NC	S3										S43
E	PC	S4										S44
F	PC	S4										S44
G	S1	S5									S41	S45
H	S1	S5									S41	S45

Scheme description: testing of 45 samples (S1-S45) by conducting the test twice. Positive control (PC), negative control (NC), test blank control (BC). PC, NC, BC are the means to assess the reliability of the test conducted. They are compulsory upon conducting each test.

13.2. Washing

Remove protective film, empty the content, subsequently wash the microplate 4 times (4 washing cycles) by adding **350 µl** of ready to use wash solution (see pt. 10).

Empty or pump out the content. The remainder of the wash solution must be removed by turning the microplates upside-down and absorbing the remaining water using a disposable towel.

The washing can be done using an automatic device.

Step 2

13.3. Incubation with enzyme conjugate

Fill each ELISA microplates with **100 µl** of ready to use (see pt. 10) enzyme conjugate. Cover the microplate with a protective foil and incubate at +37 °C for 1 hour.

13.4. Washing

Remove the protective foil. Subsequently wash the microplate 4 times (4 washing cycles) by adding **350 µl** of ready to use wash solution (see pt. 10).

Step 3

13.5. Incubation with substrate

Add **100 µl** of the enzyme substrate solution into each microplate well. Incubate the microplates at room temperature (+20°C to 25 °C) for 10 min – do not leave them in direct sunlight.

13.6. Stopping the reaction

Add **50 µl** of STOP solution into microplate wells with enzyme substrate solution.

13.7. Measuring the signal

The intensity of the colour, produced during the reaction is measured spectrophotometrically at a wavelength of 450 nm and reference wavelength at 620 nm.
The measurements must be done immediately after adding the STOP solution.

14. Evaluation of the results

During the evaluation it is necessary to consider the parameters, provided in the quality control data sheet (see the Quality Control Data Sheet). If the results obtained match the quality control OD values, continue to evaluate the results. In case of deviations from the quality control parameters, the test must be repeated.
Begin with calculating the **limit value of the test (LV):** sum the negative control OD average and the **test coefficient value 0.9.**

LV=NC OD average + 0.9

Tested sample signal assessment

The test results (relative values – RV) are calculated by dividing the OD average by the calculated limit value of the test.
RV=sample OD average/LV

Interpretation of the results:
If RV ≥ 1.0 – the result is positive
If the RV is 0.8-1.0 – the result is marginal and the sample must be repeated
If RV < 0.8 – the result is negative

<u>Quality control</u>
A test is regarded as reliable, if:
The OD average of negative controls (NC) is < 0.5
The OD average of positive controls (PC) is > 2.0
The OD average of the blank control (BC) is < 0.1

15. Description of a clinical test

The reliability and effectiveness of the **SARS-CoV-2 S IgG ELISA** test was established by conducing comparative (validation) tests with serological tests of different manufacturers. Test validation was conducted using blood plasma samples (n=210), approved by Vilnius Regional Biomedical Research Ethics Committee (No. 2020/5-N2-1231-710). The tests created were compared to CE IVD ELISA (ELISA_test_1) and serological fast tests (Fast_test_1) by testing positive blood samples from patients with PCR confirmed SARS-CoV-2 infection (n=50 samples taken 14-51 days since the onset of the symptoms (≥ 14 days) and negative blood samples, collected prior to the COVID-19 pandemic (01/06/2019) – these patients had not undergone the SARS-CoV-2 RT-PCR test (n=160). The results of the description of SARS-CoV-2 S IgG ELISA are provided in the ***Table 1.*** The sensitivity and specificity of serological diagnostic tests have been evaluated according to the RT-PCR test results.

Table 1. **SARS-CoV-2 S IgG ELISA** sensitivity and specificity evaluation.

Comparative test	RT-PCR		
Diagnostic and other serological tests	**SARS-CoV-2 S IgG ELISA**	Fast_test_1.	ELISA_test_1
Sensitivity (n=50). Correct negatives, %	**94.00**	92.00	92.00
Specificity (n=160). Correct negatives, %	**99.38**	99.38	98.75
Accuracy**	**99.11**	99.01	98.41

* – based on specific IgG antibody assessment
** – keeping in mind that the spread of COVID is 5 %, this shows a possibility that the test will correctly classify the samples into positive and negative

Upon testing 50 samples of patients, diagnosed with SARS-CoV-2 using RT-PCR method, using **SARS-CoV-2 IgG ELISA** test, three samples contained no IgG antibodies, thus the sensitivity of the test is 94 % (***Tables 1 and 1A***).

It has been established that **SARS-CoV-2 IgG ELISA** test is characterised by greater sensitivity (94.0 %) by detecting SARS-CoV-2 specific IgG antibodies than the **Fast_test_1** (considering IgG) and **ELISA_test_1** (***Table 1***).

Table 1A. **SARS-CoV-2 S IgG ELISA** sensitivity evaluation. Mismatch among samples tested.

Coded sample No.	RT-PCR	**SARS-CoV-2 S IgG ELISA**	Fast_test_1.	ELISA_test_1	Number of days since the onset of the symptoms	Days after positive RT-PCR	Age of the patient
163	1	0	0	0	51	43	63
181	1	0	0	0	44	36	63
183	1	1	0	0	14	5	60
184	1	0	0	0	40	37	54
Sensitivity (n=50). correct positives, %		94.00	92.00	92.00			

1 – positive result; 0 – negative result
* – based on specific IgG antibody assessment

It is likely that SARS-CoV-2 distribution in Lithuania in 2019 was 0 %, thus, the specificity of the **SARS-CoV-2 IgG ELISA** test was evaluated using samples (patient age 18-63) collected before 01/06/2019. The testing of the samples using **SARS-CoV-2 IgG ELISA** test showed 99.38 % specificity (***Tables 1 and 1B***). The same specificity was established using the **Fast_test_1** (IgG assessment). The specificity of **SARS-CoV-2 IgG ELISA** is higher than **ELISA_test_1** (98.75 %).

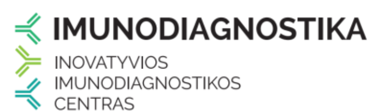
Table 1B. **SARS-CoV-2 S IgG ELISA** test specificity evaluation. Mismatch among samples tested.

Coded sample No.	RT-PCR	**SARS-CoV-2 S IgG ELISA**	Fast_test_1.	ELISA_test_1	Age of the patient
12	N	0	0	1	50
78	N	0	0	1	44
87	N	0	1	0	21
141	N	1	0	0	19
Specificity (n=160), correct negatives, %		99.38	99.38	98.75	

1 – positive result
0 – negative result
N – not tested
* – based on specific IgG antibody assessment

16. Limitations of the procedure

- Upon conducting a medical diagnosis, the result of a serological test must always be interpreted together with clinical symptoms of the patient and other test results (direct pathogen identification). For example, a negative serological test result does not negate the presence of the disease.



- Appropriate sample collection and storage are essential for the test results.
- Test validation has been conducted to identify SARS-CoV-2 IgG only in human serum or plasma.
- The antibody binding and activity of the enzyme used depends on temperature. Therefore, it is recommended to use an adjustable thermostat for the incubation. The higher the room temperature, the greater the test values. The effect of temperature on the controls is the same, thus, the changes will largely be compensated during the calculations of the results.
- Insufficient washing may increase the test values.
- The remaining washing solution in the wells may result in falsely low test values.

Literature

1. Woelfel, R. *et al.* **Clinical presentation and virological assessment of hospitalized cases of coronavirus disease 2019 in a travel–associated transmission cluster.** *medRxiv* 2020.03.05.20030502 (2020) doi:10.1101/2020.03.05.20030502.
2. Zhao, J. *et al.* **Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019.** *Clin. Infect. Dis.* (2020) doi:10.1093/cid/ciaa344.
3. OKBA, N. *et al.* **SARS-CoV-2 specific antibody responses in COVID–19 patients.** *Emerg. Infect. Dis.* 2020.03.18.20038059 (2020) doi:10.1101/2020.03.18.20038059.
4. Liu, W. *et al.* **Evaluation of Nucleocapsid and Spike Protein–based ELISAs** for Downloaded from. *J. Clin. Microbiol* (2020) doi:10.1128/JCM.00461–20.
5. Long, Q. *et al.* **Antibody responses to SARS-CoV-2 in COVID–19 patients: the perspective application of serological tests in clinical practice.** *medRxiv* 2020.03.18.20038018 (2020) doi:10.1101/2020.03.18.20038018.
6. Wan, W. Y., Lim, S. H. & Seng, E. H. **Cross–reaction of sera from COVID–19 patients with SARS–CoV assays.** *medRxiv* 2020.03.17.20034454 (2020) doi:10.1101/2020.03.17.20034454.
7. Stephan Menzel et al. **A simple low cost assay for detecting antibodies against the SARS–CoV2 Spike and nucleoprotein.** in *Half–day COVID 19 Antibodies webinar*.
8. Fafi–Kremer, S. *et al.* **Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France.** *EBioMedicine* **59**, (2020).

Legend

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic measure		Date of manufacture
CE	CE marked		Manufacturer
LOT	Product LOT number	REF	Catalogue number
2°C 8°C	Storage temperature		Biohazard
	Expiration date		

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