Anti-SARS-CoV-2 ELISA (IgG)

Instruction for use

For in vitro diagnostic use IVD

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2606-9601 G	SARS-coronavirus-2 (SARS-CoV-2)	IgG	Ag-coated microplate wells	96 x 01 (96)

CE

Intended use

The enzyme immunoassay (ELISA) provides semiquantitative in vitro determination of human antibodies of the immunoglobulin class IgG against SARS-CoV-2 in serum, EDTA, heparin or citrate plasma to support the diagnosis of SARS-CoV-2 infection and constitutes a supplement to the direct pathogen detection. Moreover, serology can be applied to collect epidemiological data. The product is designed for use as IVD. The test can be processed fully automatically.

Clinical significance

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) belongs to the family of coronaviruses and, like SARS-CoV, is classified in the genus Betacoronavirus [1]. The new coronavirus originated in China in the city of Wuhan, Hubei province. It caused an infection wave, which has spread rapidly within the country and worldwide [2, 3]. Just a few days after the first report about patients with pneumonia of unclear origin, the causative pathogen was identified as SARS-CoV-2 [2-4].

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected patients [2, 3, 5]. Health care personnel and family members are among the high-risk populations [5, 6]. The zoonotic reservoir of the virus appears to be bats [2, 5].

The incubation time of SARS-CoV is three to seven, maximally 14 days [2]. The symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties and fatigue [2, 3, 5]. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates. Some patients, especially elderly or chronically ill patients, develop severe acute respiratory distress syndrome (ARDS). The disease is fatal in around 3% of cases [2, 3, 5]. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO.

Suitable methods for diagnosis of SARS-CoV-2 infections are direct detection of the virus by polymerase chain reaction (PCR) primarily in sample material from the upper (smear) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, nasopharyngeal secretion, oropharyngeal secretion, etc) and the detection of antibodies against SARS-CoV-2 in blood. The determination of antibodies enables confirmation of SARS-CoV-2 infection in patients with typical symptoms and in suspected cases without symptoms. It also contributes to monitoring and outbreak control. For significant serological results, two patient samples should be investigated, one from the acute phase (week 1 of the illness) and one from the convalescent phase (3 to 4 weeks later) [4, 7, 8].

Cross reactions with antibodies within the genus Betacoronavirus are known [9]. Currently, there is no medication or vaccine available against infection with this new virus [2, 6].

Antigen

The microplate wells are coated with recombinant structural protein (S1 domain) of SARS-CoV-2.

Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with recombinant structural protein of SARS-CoV-2. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG (also IgA and IgM) antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	-	12 x 8	STRIPS
2. Calibrator (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgG, ready for use	green	1 x 12 ml	CONJUGATE
6. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	-	1 booklet	-
11. Quality control certificate	-	1 protocol	-
12. Protective foil	-	3 pieces	FOIL

Additional materials and equipment (not supplied in the test kit)

- Automatic microplate washer: recommended. Washing of the microplates can also be carried out manually.
- Microplate reader: wavelength of 450 nm, reference wavelength range from 620 nm to 650 nm
- Calibrated pipettes
- Pipette tips
- Stepper pipette: recommended for the pipetting of enzyme conjugate, substrate, and stop solution
- Distilled or deionised water
- Incubator: for incubation of the microplate at +37°C
- Incubator or water bath: recommended to warm the wash buffer
- Stop watch

Storage and stability

The test kit has to be stored at a temperature between +2°C and +8°C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

In use stability following the first opening

After opening, the reagents are stable until the indicated expiry date when stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

Warnings and precautions

- The product must only be used by trained laboratory staff in a clinical or research laboratory.
- If the packed reagents are visibly damaged, do not use the test kit.
- Before using the product, read the instruction for use carefully. Use only the valid version provided with the product.
- The pipetting volumes, incubation times, temperatures, and preparation steps given in the instruction for use must be adhered to.
- Do not substitute or mix the EUROIMMUN reagents with reagents from other manufacturers.
- Observe Good Laboratory Practice (GLP) and safety guidelines. Some of the reagents contain preservatives in non-declarable concentrations. Avoid eye and skin contact with samples and reagents. In case of eye or skin contact, rinse thoroughly with water. Remove and wash contaminated clothing. In case of ingestion, obtain medical advice.
- The calibrator and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all reagents should be treated as being a potential infection hazard and should be handled with care.

Preparation and stability of the samples

- **Samples:** Human serum or EDTA, heparin or citrate plasma.
- **Sample preparation: Patient samples** are diluted 1:101 in sample buffer.

  For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).
- **Stability of the patient samples:**
  - stored at +2°C to +8°C: up to 14 days
  - incubate diluted samples within one working day

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use.

The thermostatically adjustable ELISA incubator must be set to +37°C ± 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antibodies can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrator and controls:** Ready for use. Mix reagents thoroughly before use.
- **Enzyme conjugate:** Ready for use. Mix the reagent thoroughly before use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before dilution. The quantity required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water (1 part reagent plus 9 parts water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable until the indicated expiry date if stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready to use.

Waste disposal

Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Quality control

For every group of tests performed, the extinction readings of the calibrator and ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Reference material

As no quantified international reference serum exists for antibodies against SARS-CoV-2, the calibration is performed in ratios which are a relative measure for the concentration of antibodies in serum or plasma.

Assay procedure

(Partly) manual test performance

Sample incubation: (1st step)
Transfer **100 µl** of the **calibrator, positive and negative controls or diluted patient samples** into the individual microplate wells according to the pipetting protocol. Incubate for **60 minutes** at **+37 °C ± 1 °C**.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation follow the recommendations of the instrument manufacturer.

Washing:
Manual: Remove the protective foil. Empty the wells and subsequently wash **3 times using 300 µl of working-strength wash buffer** for each wash.
Automatic: Remove the protective foil. Wash the reagent wells **3 times with 450 µl of working-strength wash buffer** (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note:
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette **100 µl of enzyme conjugate** (peroxidase-labelled anti-human IgG) into each of the microplate wells. For manual test performance cover the reagent wells with the protective foil.
Incubate **30 minutes at +37°C ± 1°C**.

Washing:
Remove the protective foil. Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette **100 µl of chromogen/substrate solution** into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C) protected from direct sunlight.

Stopping:
Pipette **100 µl of stop solution** into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I and the EUROIMMUN Analyzer I-2P and this EUROIMMUN ELISA. Validation documents are available on enquiry.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	P 6	P 14	P 22								
B	pos.	P 7	P 15	P 23								
C	neg.	P 8	P 16	P 24								
D	P 1	P 9	P 17									
E	P 2	P 10	P 18									
F	P 3	P 11	P 19									
G	P 4	P 12	P 20									
H	P 5	P 13	P 21									

The pipetting protocol for microplate strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient sera (P 1 to P 24).

The calibrator (C), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Test evaluation

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0,8: negative
Ratio ≥ 0.8 to <1.1: borderline
Ratio ≥1.1: positive

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

Analytical performance

Measurement range: .

Limit of blank (LoB): ratio 0.13
Limit of detection (LoD): ratio 0.15

LoB and LoD were defined according to the requirements defined in guideline EP17-A2 of the CLSI (Clinical and Laboratory Standards Institute, https://clsi.org/).

Precision: Studies on the intra-lab precision were carried out according to CLSI guideline EP05-A3. Four samples (reactivity distributed over the entire measurement range) were measured. The precision is given as standard deviation (SD) and coefficient of variation (CV).

Intra-lab precision

	Sample 1		Sample 2		Sample 3		Sample 4	
Mean	Ratio 0.07		Ratio 1.12		Ratio 2.36		Ratio 5.20	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Repeatability	0.012	16.0	0.060	5.4	0.091	3.9	0.231	4.4
Between run	0.000	0.0	0.021	1.9	0.058	2.4	0.168	3.2
Within day	0.012	16.0	0.063	5.7	0.108	4.6	0.285	5.5
Between day	0.002	2.3	0.060	5.4	0.174	7.4	0.089	1.7
Within lab	0.012	16.2	0.087	7.8	0.205	8.7	0.299	5.7

Cross-reactivity (analytical specificity): Due to low homologies of the S1 protein within the coronavirus family, cross-reactions to most of the human pathogenic representatives of this virus family are virtually excluded. However, due to the close relationship of SARS-CoV(-1) and SARS-CoV-2, cross-reactions between these two viruses are likely. Sera from patients with SARS-CoV(-1), MERS-CoV, HCoV-229E, HCoV-NL63, HCoV-HKU1 or HCoV-OC43 infections were investigated to examine this further. Pronounced cross-reactions occur mainly with Anti-SARS-CoV(-1) IgG antibodies. Cross-reactions to other human pathogenic coronaviruses were not observed.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to concentrations of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA.

Clinical performance

Diagnostic sensitivity: The sensitivity was determined by investigating 9 samples from 8 European patients, using the Anti-SARS-CoV-2 ELISA (IgA) and the Anti-SARS-CoV-2 ELISA (IgG). In these patients, infections with SARS-CoV-2 had been confirmed by RT-PCR test [4] based on one sample each, taken at the early phase of infection. The serological test was performed on samples taken at a later stage of infection. The tables show the results with respect to specific antibodies of classes IgA and IgG. The determined sensitivities are shown in two groups, i.e. the early samples (< 10 days after onset of symptoms) and the later samples (> 10 days after onset of symptoms). The two lines marked in grey show two samples from one patient over the course of time.

Sample	Days after symptom onset	Disease severity	EUROIMMUN Anti-SARS-CoV-2 ELISA			
			IgA (ratio)	**IgA**	**IgG** (ratio)	**IgG**
			pos.: ≥ 1.1	Results	**pos.: ≥ 1.1**	Results
			borderl.: 0.8 – 1.0		borderl.: 0.8 – 1.0	
< 10 days after onset of symptoms						
1	**4**	**mild**	0.2	neg.	0.1	neg.
2	**7**	**severe**	**7.2**	**pos.**	**4.4**	**pos.**
3	**8**	**severe**	**2.0**	**pos.**	0.3	neg.
4	**8**	**severe**	0.2	neg.	0.8	borderl.
> 10 days after onset of symptoms						
5	**13**	**mild**	**2.3**	**pos.**	0.3	neg.
6	**13**	**mild**	**2.1**	**pos.**	**1.3**	**pos.**
7	**16**	**mild**	**8.5**	**pos.**	**6.7**	**pos.**
8	**18**	**mild**	**2.7**	**pos.**	**1.9**	**pos.**
9	**32**	**mild**	**1.8**	**pos.**	**1.1**	**pos.**

Group	EUROIMMUN Anti-SARS-CoV-2 ELISA IgG			
	positive	**borderline**	**negative**	**Sensitivity**
< 10 days after onset of symptoms	1	1	2	**33.3%**
> 10 days after onset of symptoms	4	0	1	**80.0%**

Group	EUROIMMUN Anti-SARS-CoV-2 ELISA IgA & IgG combined			
	positive	**borderline**	**negative**	**Sensitivity**
< 10 days after onset of symptoms	2	1	1	**66.7%**
> 10 days after onset of symptoms	5	0	0	**100%**

Specificity: To evaluate the specificity of the Anti-SARS-CoV-2 ELISA (IgG), a study was performed on patient sera which were positive for different viral antibodies, rheumatoid factors or diverse antibodies, as well as from patients with acute bacterial pneumonia. Out of the total of 200 samples, 3 sera were positive in the Anti-SARS-CoV-2 ELISA (IgG). This panel yielded a specificity of 98.5%. The results are shown in the following table.

Possible influencing factors	**n**	**Positive result with EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)**
Acute EBV infection	22	0%
Diverse autoantibodies	40	2.5% (1 positive result)
Rheumatoid factors	40	0%
Ab against influenza (vaccines)	58	3.4% (2 positive results)
Acute bacterial pneumonia	40	0%

Prevalence: SARS-CoV-2 was first described in December 2019 as a cause for infections in the Chinese population. The expected prevalence values for the European panels from 2010, 2017 and 2019 therefore amount to 0%. The determined positive results correspond to a specificity of the Anti-SARS-CoV-2 ELISA (IgG) of 99%. The following prevalence values were determined in apparently healthy blood donors and children from 3 to 10 years of age:

Panel	**n**	**Positive result with EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)**
Blood donors (2010)	150	1.3%
Blood donors (2017)	250	0.8%
Children (3 – 10 years, Oct. 2019)	100	1.0%

Limitations of the procedure

- For a medical diagnosis, the serological test result should always be interpreted together with the clinical symptoms of the patient and other results, e.g. those of the direct pathogen detection. A negative serological test result does not exclude the presence of the disease.
- Correct performance of sample collection and storage is crucial for the test results.
- The test system is validated for the determination of anti-SARS-CoV-2 IgG in human serum or plasma only.
- The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostatically adjusted ELISA incubator in all incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction. The same variations also apply to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
- Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.
- Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.
- The partial or complete adjustment of the test system to the use of instruments for automated sample processing or other liquid handling devices may result in differences between the results obtained with automated processing and those obtained with manual procedure. It is the responsibility of the user to validate the instruments used so that they yield test result within the reliable range.

Literature

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Technical support

In case of technical problems you can obtain assistance via the EUROIMMUN website (www.euroimmun.com/contact).

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips		Protect from sunlight
CAL	Calibrator		Storage temperature
POS CONTROL	Positive control		Unopened usable until (YYYY-MM-DD)
NEG CONTROL	Negative control		CE-labelled
CONJUGATE	Enzyme conjugate		Manufacturing date (YYYY-MM-DD)
SAMPLE BUFFER	Sample buffer		Manufacturer
WASH BUFFER 10x	Wash buffer, 10x concentrate		Observe instructions for use
SUBSTRATE	Substrate	REF	Order number
STOP SOLUTION	Stop solution		Contents suffice for <n> analyses
IVD	In vitro diagnostic medical device		Biological risks
LOT	Lot description		