SARS-CoV-2 Antigen ELISA
Instruction for use

For in vitro diagnostic use IVD

ORDER NO.	ANTIGEN	SUBSTRATE	FORMAT
EQ 2606-9601	Nucleocapsid protein of SARS coronavirus 2 (SARS-CoV-2)	Ab-coated microplate wells	96 x 01 (96)

CE

Intended use

The SARS-CoV-2 Antigen ELISA is a semiquantitative enzyme immunoassay for in vitro determination of SARS-CoV-2 nucleocapsid protein in human sample material from the upper respiratory tract (nasopharyngeal swabs). It supports the diagnosis of acute SARS-CoV-2 infections. This product is only intended for use by medical professionals and can be performed and evaluated automatically or manually. The interpretation of results should always be based on further laboratory diagnostic methods as well as the clinical picture. The product is designed for use as IVD.

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]. At the end of 2019, SARS-CoV-2 was identified as the causative pathogen in a cluster of pneumonia cases of unclear origin. The virus caused an infection wave that has spread rapidly over the world and was declared a pandemic by the WHO at the beginning of 2020 [2-5].

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons [3-4, 6]. Healthcare personnel and family members are especially at risk of infection [6]. The zoonotic reservoir of the virus appears to be bats [3, 4, 6].

The incubation time of SARS-CoV-2 is three to seven, maximally 14 days [2]. The symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties and fatigue [2-4, 6]. 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 acute respiratory distress syndrome (ARDS) [2, 3, 5, 6]. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO.

Suitable methods for the diagnosis of SARS-CoV-2 infections are detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or of viral protein by ELISA primarily in sample material from the upper (nasopharyngeal or oropharyngeal swabs) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, etc.). The detection of viral antigens is less sensitive than RT-PCR.

The determination of antibodies enables confirmation of SARS-CoV-2 infection in patients with typical symptoms and in suspected cases. It also contributes to monitoring and outbreak control [4, 5]. The spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 are highly immunogenic. Antibodies against receptor binding domain (RBD) of the spike protein are neutralising [7]. 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, 6, 8]. Cross-reactions with antibodies within the genus *Betacoronavirus* have been described [4, 5].

The majority of COVID-19 patients in the convalescent phase show a pronounced T-cell reactivity to SARS-CoV-2. Cross-reacting T-cells are found in around half of the patients without SARS-CoV-2 history. This is presumably due to past infections with coronaviruses causing common colds. SARS-CoV-2-specific T-cells are part of the T-cell repertoire of persons who had an infection with SARS-CoV in 2003. These cells proliferate following contact with SARS-CoV-2. These findings indicate a long-term immunity after infection with Betacoronaviruses [9].

Antibody

The microplate wells are coated with a monoclonal anti-SARS-CoV-2 antibody.

Test principle

The test kit contains microplate strips each with 8 reagent wells coated with a monoclonal anti-SARS-CoV-2 antibody. The patient samples are inactivated by lysis of the virus before pipetting them into the reagent wells in the first incubation step. In the second incubation step, biotinylated anti-SARS-CoV-2 antibody is added to the sample which is then detected by streptavidin-bound horseradish peroxidase. The colour intensity is proportional to the SARS-CoV-2 antigen concentration in the sample.

Contents of the test kit

Component	Colour	Format	Symbol
1. Antibody-coated microplate wells 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	-	12 x 8	STRIPS
2. Calibrator, ready for use	light red	1 x 2.0 ml	CAL
3. Positive control, ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control, ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Biotin biotin-labelled anti-SARS-CoV-2 antibody, ready for use	green	1 x 12 ml	BIOTIN
6. Enzyme conjugate peroxidase-labelled streptavidin, ready for use	blue	1 x 12 ml	CONJUGATE
7. Sample buffer, ready for use	colourless	1 x 12 ml	SAMPLE BUFFER
8. Lysis buffer, ready for use	cloudy	1 x 100 ml	LYSIS BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	-	1 booklet	-
13. Quality control certificate	-	1 protocol	-
14. 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 sample buffer, biotin, enzyme conjugate, substrate, and stop solution
- Distilled or deionised water
- Stop watch
- Glass/polypropylene tubes with screw cap (for example, 2-ml Sarstedt tubes without insert): recommended for sample preparation
- +37°C incubator or water bath: recommended to warm the lysis buffer and wash buffer

Storage and stability

The test kit has to be stored at a temperature between +2°C to +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 personnel 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.
- Do not substitute or mix the EUROIMMUN reagents with reagents from other manufacturers.
- Wash buffers, substrate and stop solutions are exchangeable between lots if they have identical article numbers (see labelling). The product-specific lysis buffer and sample buffer are also exchangeable between lots. All other reagents are lot-specific, which means that they must not be combined with reagents of other lots.
- When handling the samples and reagents, laboratory coat, disposable gloves and, if required, further personal protective equipment should be used. The valid recommendations for the SARS-CoV-2 testing, such as those from the German Committee for Biological Work Substances (ABAS) or the World Health Organization (WHO) should be observed.
- 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.

Preparation and stability of the samples

- **Samples:** Human sample material from the upper respiratory tract (nasopharyngeal swabs).

  Note: The medium of swabs used must not be denaturing; media based on guanidine thiocyanate are not suitable. The Robert Koch Institute (Germany) advises against using agar swabs [10].

  Recommendation: e.g. Viral Transport Media (VTM), Universal Transport Media (UTM), NaCl.

- **Sample preparation:**

  The samples to be investigated have to be diluted 1:1.5 with lysis buffer (e.g. 1 ml sample + 0.5 ml lysis buffer), mixed and incubated at least 5 minutes at room temperature. This step lyses the virus in the sample.

- **Stability of the patient samples:**
  - untreated samples stored at +2°C to +8°C: up to 7 days
  - lysed samples stored at +2°C to +8°C: up to 3 days

Note: The calibrator and the controls are ready for use, do not dilute them.

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.

- **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:** Ready for use. Mix calibrator thoroughly before use.
- **Controls:** Ready for use. Mix controls thoroughly before use.
- **Biotin:** Ready for use. Mix biotin thoroughly before use.
- **Enzyme conjugate:** Ready for use. Mix enzyme conjugate thoroughly before use.
- **Sample buffer:** Ready for use.
- **Lysis buffer:** Ready for use. Mix lysis buffer 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 diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable for 4 weeks when 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 for use.

Waste disposal

Patient samples, calibrator, 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 reading of the calibrator and the ratio values determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate with the respective data is provided. If the values specified for the

calibrator and the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Reference material

Since there is no qualified international reference material for the determination of SARS-CoV-2 nucleocapsid protein, the results are given in ratio values.

Assay procedure

(Partly) manual test performance

Sample incubation: (1st step)
Pipette **100 µl** of **sample buffer** into each reagent well and, according to the pipetting scheme, pipette **50 µl of calibrator, positive and negative control** or **prepared patient sample**. Cover the finished test plate with the protective foil.
Incubate for **1 hour at room temperature** (+18°C to +25°C).

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 and 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.

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.

Biotin incubation: (2nd step)
Pipette **100 µl** of **biotin** into each of the microplate wells.
Cover the wells.
Incubate for **1 hour at room temperature** (+18°C to +25°C).

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

Enzyme conjugate incubation: (3rd step)
Pipette **100 µl** of **enzyme conjugate** into each of the microplate wells.
Cover the wells and Incubate for **1 hour at room temperature** (+18°C to +25°C).

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

Substrate incubation: (4th step)
Pipette **100 µl** of **chromogen/substrate solution** into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C; protect 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, carefully shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

The preparation of samples and controls is performed manually; the subsequent test procedure can be performed on an automated analysis instrument. 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.

Note: Processing on other fully automated systems is possible but must be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	Pa 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 single determinations. 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.

The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.

Test evaluation

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:

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

Two digits after the decimal point should be indicated. EUROIMMUN recommends interpreting results as follows:

Ratio <0.50: negative
Ratio ≥0.50 to <0.60: borderline
Ratio ≥0.60: 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 0.36
Limit of detection (LoD): ratio 0.45

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: Six 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		Sample 5		Sample 6	
Mean	Ratio 0.34		Ratio 0.61		Ratio 1.24		Ratio 2.26		Ratio 3.92		Ratio 6.43	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	0.033	9.9	0.035	5.8	0.073	5.9	0.099	4.4	0.185	4.7	0.274	4.3
Between run	0.008	2.2	0.036	6.0	0.064	5.1	0.165	7.3	0.283	7.2	0.531	8.3
Within day	0.034	10.1	0.051	8.3	0.097	7.8	0.192	8.5	0.338	8.6	0.597	9.3
Between day	0.019	5.7	0.039	6.5	0.074	6.0	0.133	5.9	0.149	3.8	0.477	7.4
Within lab	0.039	11.6	0.064	10.5	0.122	9.8	0.234	10.4	0.370	9.4	0.765	11.9

Cross-reactivity: SARS-CoV-2-negative nasopharyngeal swabs were treated with 1 µg/ml of recombinant nucleocapsid protein of human pathogenic coronaviruses. The determined cross-reactivity is shown in the table below.

Cross-reactant	**Result**
SARS-CoV-1	pos.
229E	neg.
NL63	neg.
OC43	neg.
HKU1	neg.
MERS	neg.

***Interference*:** SARS-CoV-2-negative and -positive nasopharyngeal swabs were treated with potentially interfering viruses and reagents. The determined interference is shown in the table below.

	Concentration	**Interference**
Microbial interference		
Influenza virus type A	n. d.*	negative
Influenza virus type B	n. d.*	negative
RSV type B (ATCC)	10^4 PFU/ml	negative
Endogenous interference		
Biotin	120 µg/ml	negative
Whole blood	0.5% (v/v)	negative

*n. d.: Panel confirmed by molecular diagnostic testing

Clinical performance

Positive and negative agreement

The clinical performance of the EUROIMMUN SARS-CoV-2 Antigen ELISA was determined using a total of 98 nasopharyngeal swabs. 48 samples were from symptomatic patients (sample collection < 10 days after symptom onset) with SARS-CoV-2 infections confirmed by molecular diagnostic testing. 50 samples originated from patients with influenza virus infections confirmed by molecular diagnostic testing. The samples were collected during the COVID-19 pandemic in 2020. After collection, the samples were stored at -20°C and lysed before measurement according to the instructions using the lysis buffer provided in the test kit. Borderline results were not included in the calculation.

	n = 98	Molecular diagnostic test	
		positive	negative
EUROIMMUN SARS-CoV-2 Antigen ELISA	positive	44	0
	borderline	1	0
	negative	3	50

Positive agreement (sensitivity): 93.6 % (95% confidence interval: 82.5 % – 98.7 %)
Negative agreement (specificity): 100 % (95% confidence interval: 92.9 % – 100 %)

Limitations of the procedure

- The result of this test is no proof of the presence or absence of a disease. The test kit is used to support diagnostics. The results should always be interpreted together with clinical findings and further diagnostic tests.
- A negative result does not exclude an infection with SARS-CoV-2. Especially in early phases of infection, antigen may not be present or only be present in undetectable amounts in the nasopharyngeal cavity. With suspected clinical cases and negative or borderline results, clarification by means of molecular diagnostic methods and/or the analysis of a subsequent sample is recommended.
- The pipetting volumes, incubation times, temperatures, and preparation steps given in the instructions must be adhered to.
- Correct sample collection and storage is crucial for the test results.
- The test system was not validated with samples of infants.
- 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 substrate incubation, the greater will be the extinction values. However, the calibrator is 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 reaction times) can lead to false extinction readings.
- Residual liquid (>10 µl) remaining 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 on instruments for automated sample processing or other liquid handling devices may lead to differences between the results obtained by the automated and by the manual procedure. It is the responsibility of the user to validate the instruments used so that they yield test results within the permitted range.
- Nasopharyngeal swabs visibly contaminated with blood should not be used.

Literature

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Technical support

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

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips	LOT	Lot description
CAL	Calibrator	IVD	In vitro diagnostic medical device
BIOTIN	Biotin		Storage temperature
POS CONTROL	Positive Control		Unopened usable until (YYYY-MM-DD)
NEG CONTROL	Negative Control	CE	CE-labelled
CONJUGATE	Enzyme conjugate		Protect from sunlight
SAMPLE BUFFER	Sample buffer		Manufacturing date (YYYY-MM-DD)
LYSIS BUFFER	Lysis 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
FOIL	Protective foil		Biological risks