Microgen Bioproducts® SARS-CoV-2 IgG EIA

Instructions for use

IVD In-vitro Diagnostic Medical Device

REF MBCOV2CE

480 Determinations

Storage at 2-8°C

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MICROGEN BIOPRODUCTS

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English

Please read the instructions for use carefully before performing the test. Be sure to follow the instructions of this product insert. Any deviation from these instructions for use (IFU) may cause unreliable results for which the manufacturer is not liable.

1. Product description

Introduction

SARS-CoV-2 belongs to the *Coronaviridae* family and is closely related to the SARS coronavirus that appeared in 2002/2003 [1]. The virus was first identified in humans in Wuhan, China, at the end of 2019 and is transmissible from person to person [2]. The main route of transmission is droplet infection, but infections via aerosols and smear have also been described [2-4]. The incubation period is usually three to seven days, up to a maximum of fourteen days [2]. SARS-CoV-2 infected patients are often asymptomatic or experience only mild symptoms such as a dry cough, fever, and shortness of breath [3,4]. Some of the infected persons develop a severe pneumonia, which can lead to death.

The virus can be diagnosed by reverse transcription polymerase chain reaction (RT-PCR) from upper and lower respiratory tract samples (pathogen detection) [5]. An indirect ELISA can also be used to detect antibodies against SARS-CoV-2 to confirm the infection, to monitor the course of the disease and to draw epidemiological conclusions [3]. Detectable antibodies are usually formed two weeks (>14 days) after initial infection [6].

Intended use

The Microgen Bioproducts® SARS-CoV-2 IgG ELISA is intended for the qualitative detection of specific IgG antibodies against SARS- CoV-2 virus in human serum or plasma (EDTA, citrate, NaF). The test supports the diagnosis of a SARS-CoV-2 infection and is a supplement to the pathogen's initial detection. It can be used by health authorities to collect epidemiological data, for example to investigate the immune status of the population. The product is intended for use as an IVD.

The present test procedure has been validated for manual application, however, is also suitable for high-throughput ELISA automation.

Test principle

The test principle is based on the binding of SARS-CoV-2 specific antibodies to the antigen immobilized on the microtiter plate. Unbound antibodies are removed by washing after this first step. During the second incubation phase, enzyme-labelled secondary antibodies directed against human IgG class antibodies bind to the antigen-antibody complex. Unbound secondary antibodies are removed by washing again. For detection, a colourless substrate solution is added, which is converted to a blue dye by the enzyme of the secondary antibody. In the last step, the reaction is stopped and at the same time the colour changes from blue to yellow. The absorbance of the solution is read and correlates to the initially bound antibodies against the antigen.

Limitations of the procedure

The IgG antibodies to be detected are produced on average 10 to 14 days after a person has been infected with SARS-CoV-2. Sampling before this period may give a negative result, even though the patient had an infection.

Immunocompromised patients may also fail to develop an antibody response.

To the present day there exists no scientifically conclusively proof as to how long antibodies are present after infection with SARS-CoV-2 and how far developed antibodies provide immunity. The test is not suitable for diagnosing an acute infection with SARS-CoV-2. The test is designed only for single samples and not for pools of samples. The test does not allow conclusions to be drawn about the antibody concentration in the sample.

2. Components of the kit

Table 1: Overview of the components

Components	Colour	Symbol	Quantity
Microplate coated with SARS-CoV-2 antigen *ready to use*	Plate in silver-grey wrapping	STRIPS	5 x 96 well plates
Positive control (PC) *ready to use*	Red reagent and cap	POS CONTROL	1 x 3.5 mL
Negative control (NC) *ready to use*	Blue reagent and cap	NEG CONTROL	1 x 3.5 mL
Wash buffer *10x concentrate*	Colourless buffer	WASH BUFFER 10x	2 x 125 mL
Sample buffer *ready to use*	Green buffer	SAMPLE BUFFER	2 x 125 mL
Conjugate *ready to use*	Orange reagent	CONJUGATE	1 x 60 mL
Substrate solution *ready to use*	Brown bottle with brown cap	SUBSTRATE	1 x 60 mL
Stop solution *ready to use*	Yellow dot on the cap	STOP SOLUTION	1 x 60 mL
Instructions for Use *ready to use*			1

3. Materials required but not supplied

- Manual or automated microplate washer system
- Microplate reader, equipped for the measurement of absorbance at 450/620nm
- Micropipettes (1 – 1,000 µL)
- Vortex mixer
- Distilled or deionized water (conductivity ≤ 5 µS/cm)
- Disposable plastic test tubes

4. Storage and stability

After being delivered at room temperature, the kits need to be stored at 2 - 8°C. When stored under these conditions, the kit maintains quality, efficiency, and stability up to the expiration date stated on the label.

5. Sample material and stability

Sample material:

- Human serum or plasma (citrate, EDTA, NaF) samples.
- Shipment including packaging and labelling according to national and international guidelines.

Stability:

- Samples are stable for up to 48 hours at 2 to 8°C; for longer periods, its recommended samples are aliquoted and stored at < -15°C. Avoid repeated freeze-thaw cycles.
- Thawed samples need to be thoroughly mixed prior to use.
- Do not heat-inactivate samples.

Sample dilution:

- Before the test, dilute samples 1:100 with SAMPLE BUFFER. E.g. dispense 5 µL of the sample with 495 µL of the SAMPLE BUFFER and thoroughly mix with a vortex.
- Diluted patient samples need to be used right away and should not be stored.

6. Preparation

Wash buffer:

- The WASH BUFFER 10x must be diluted 1 to 10 prior to use. E.g. 100 mL of the WASH BUFFER 10x + 900 mL of water.
- The diluted buffer can be stored for up to 14 days at 2 to 8°C.
- If the WASH BUFFER 10x shows crystal formation, warm up the solution to 37 °C. E.g in a water bath, mix thoroughly before diluting.

7. Test procedure

Please read the instructions for use carefully before performing the ELISA test and be sure to follow the instructions closely. The procedure described below is validated for manual application.

Exactly define the microplate layout (how your samples and controls are arranged on the microplate) prior to beginning the test. Perform all test steps in the order given and without any delays; use clean, disposable tips for each pipetting step for controls and samples.

In order to verify and evaluate the test, the controls contained in the kit (positive and negative controls – *ready to use*) should be analysed in each run (see also below 'Section 8 – Calculation of Results').

Before the test, all reagents should be equilibrated to room temperature, dilute wash buffer, switch on all equipment (e.g. microplate washer & microplate reader) and prepare the test materials.

1. Add 100 µL of the POS CONTROL, NEG CONTROL and the pre-diluted samples into the appropriate wells according to the microplate layout which has been defined.
2. Incubate the plate at room temperature (20 – 25°C) for **45 minutes**.
3. After incubation discard or aspirate the incubated controls/samples from the wells (microplate washer). Wash three times by adding 300 µL of the WASH BUFFER 10x. Avoid overflow of the

wash buffer from any of the wells. The interval between “washing” and “aspiration” should be >5 seconds. After the last wash, remove any remaining fluid by tapping on the microplate with an absorbent tissue.
It is important to use the appropriate washing procedure; insufficient washing could cause inaccuracy and invalid results!

4. Add 100 µL of the CONJUGATE into each well.
5. Incubate the plate at room temperature for **30 minutes**.
6. Repeat the wash cycle as described in step 3.
7. Add 100 µL of the SUBSTRATE into each well.
8. Incubate the plate at room temperature for **10 minutes**. A blue colour occurs due to the enzymatic reaction.
9. Add 100 µL of the STOP SOLUTION into each well in the same order, and at the same intervals as for the TMB substrate solution. The addition of the stop solution converts the blue colour of the substrate, to a yellow colour.
10. Read the absorbance of the solution in each well at 450/620 nm within 30 minutes after adding the stop solution.

8. Calculation of results

Validity of results

Test results are valid if the following criteria are met:

$OD_{450/620nm}$ Negative control: <0.30

$OD_{450/620nm}$ Positive control: >1.00

If the quality criteria are not met, the test has to be repeated.

Interpretation of results

The results are interpreted with a S/P ratio obtained using the absorption values with the following formula:

S/P = (sample – NC) / (PC – NC) * 100%

The results can be interpreted as follows:

Table 2: Interpretation of results.

S/P-Ratio	Interpretation
< 20%	Negative
20 – 30%	Borderline
> 30%	Positive

In case of a borderline result, no definite interpretation is possible. It is recommended to take another sample 1 – 2 weeks after the first one and to repeat the test.

9. Analytical performance characteristics

Analytical specificity

Interferences

Increased concentration levels of haemoglobin, bilirubin and triglycerides in serum and plasma samples may influence the results of immunoassays. No effects on the test could be observed for any of the substances listed in Table 3 up to the concentrations given.

Table 3: Interferences.

Interfering substance	Concentration
Haemoglobin	< 20 mg/mL
Bilirubin	< 0.3 mg/mL
Triglycerides	< 15 mg/mL

Table 4: Cross reactivity.
n = number of samples; HIV = human immunodeficiency virus; hCoV = human coronavirus.

Pathogen/Disease	n	Positive
HIV 1/2	4	0
Parvovirus B19	4	0
Hepatis A virus	4	0
Hepatitis B virus	8	0
Cytomegalovirus	2	0
Epstein-Barr virus	2	0
Herpes simplex virus	2	0
hCoV-HKU1	1	0
Influenza A	4	0

Precision

Intra-assay precision

The study of intra-assay precision was performed in compliance with CLSI guidelines AP05-A3 by using four samples covering the entire measurement range (see Table 5).

Table 5: Intra-assay precision (coefficients of variation given in %).

Sample	Repeatability	Between Run	Within Day	Between Day	Within Lab
High-positive	1.5%	4.2%	3.2%	3.0%	4.4%
Mid-positive	3.0%	4.6%	4.0%	3.8%	5.3%
Low-positive	2.0%	6.7%	3.6%	6.1%	6.8%
Negative	4.4%	8.5%	8.0%	5.2%	9.4%

The acceptance criteria of the coefficients of variation with ≤ 10% were met in all cases.

Lot-to-lot variance

A sample set consisting of a high, medium, and low positive samples were tested on five consecutive days with five replicates, conducted using three different kit lots (see Table 6).

Table 6: Lot-to-lot variance.

SD = standard deviation; CV = coefficient of variation.

Sample	Mean	SD	CV
High Positive	1.77	0.16	9.0%
Middle Positive	1.20	0.11	9.4%
Low Positive	0.75	0.07	9.8%

The acceptance criteria of the coefficients of variation of ≤ 15% were met in all samples.

10. Clinical performance characteristics

Category	Specification	Value
Diagnostic Sensitivity[1]	< 7 days after PCR diagnostics	38.1% (8/21)
	7 – 14 days after PCR diagnostics	84% (21/25)
	> 14 days after PCR diagnostics	**100% (66/66)**
Diagnostic Specificity[1]	**SARS-CoV-2 [-]**	**99.4% (1543/1561)**
	Tumour patients[2]	100% (61/61)
	COPD patients[2]	98.9% (273/276)
	Non-smokers[2]	99.6% (676/684)
	Light smokers[2]	99.1% (451/457)
	Heavy smokers[2]	99.2% (355/359)
	Men[2]	99.0% (699/710)
	Women[2]	99.6% (783/790)

[1]Borderline results were considered negative.

[2]Overlapping subgroup within the total number of samples.

11. Precautions and warnings

- This kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the in-vitro-diagnostic procedures.
- According to the In-Vitro-Device Directive (IVDD) 98/79/EC, article 1, point 2B. The manufacturer defines the intended purpose for their in vitro medical device; to ensure its safety, quality, and performance. For this reason, the test procedure, information, precautions, and warnings within the instructions for use should be strictly followed. If performing the test on ELISA automated systems, the test method should be validated. Any change in design, composition, and test procedure as well as any use in combination with other products not approved by the

manufacturer is not authorised; the user us solely responsible for such changes. The manufacturer is not liable for incorrect results and for potential harm caused by such changes. The Manufacturer is not liable for any incorrect results due to visual evaluation of the samples.

- For In-Vitro-Diagnostic use only.
- Do not mix components from different kit lots or use reagents from other manufacturers.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Add reagents carefully to the wells in order to avoid cross-contamination and invalid results.
- Use only clean tubes and disposable tips.
- Avoid foam formation in all reagents and samples.
- Do not expose to direct sunlight whilst performing the assay.
- After use, disposable tips, tubes, strips and sample material should be disposed in accordance with national legislation.
- Do not use the test kit after the expiration date.
- All components of human origin used in the kit reagents have been tested and confirmed negative for anti-HIV antibodies, anti-HCV antibodies and HBsAg.
- All materials of human origin should be handled as though they contain infections agents. Perform each step working according to good laboratory practice (GLP).

12. References

1. Gorbalenya et al., The species Severe acute respiratory syndrome-related coronavirus: classifying 2019- nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536–44.
2. Gralinski & Menachery. Return of the coronavirus: 2019-nCoV. Viruses. 2020;12(2):1–8.
3. Udugama et al., Diagnosing COVID-19: The Disease and Tools for Detection. ACS Nano. 2020;14(4):3822–35.
4. Xiao et al., Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020;92(5):464–7.
5. WHO. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Interim Guide [Internet]. 2020:1–7. Available from: https://www.who.int/publications-detail/laboratory-tes- ting-for-2019-novel-coronavirus-in-suspected-human-cases-20200117
6. Okba et al., Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Corona- virus Disease 2019 Patients. Emerg Infect Dis. 2020;26(7):1478–88.

13. Symbols

SYMBOL	DEFINITION
	Batch Code/Lot Number
	Contains sufficient for ‘n’ tests
	In Vitro Diagnostic Medical Device
	Catalogue Reference
	Upper and Lower Temperature Limits
	Expiry Date
	Manufacturer and Address Details
	Date of Manufacture
	Please consult IFU
	Sufficient for
	Do not use if package is damaged
	CE Mark
	Caution

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