Manual

IDK® anti-SARS-CoV-2 IgM ELISA

For the qualitative in vitro determination of human IgM antibodies against SARS-CoV-2 in EDTA plasma and serum

Valid from 2020-08-25

REF **K 5001** Σ 96 +8 °C +2 °C IVD CE

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1. INTENDED USE

IDK® anti-SARS-CoV-2 IgM is an enzyme-linked immunosorbent assay (ELISA) for qualitative measurement of IgM antibodies against the novel coronavirus SARS-CoV-2 in human serum or EDTA plasma. The assay is an *in vitro* diagnostic medical device and is intended to be used by professional users in a laboratory environment. This ELISA can be performed manually or using an automated platform. This test serves as complement to direct infection diagnosis and provides evidence for a beginning immunoreaction against SARS-Cov-2.

2. INTRODUCTION

The virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is an enveloped, single stranded RNA virus and is a family member of the coronavirus family *Coronaviridae* [1,2].

Coronaviruses have a similar composition as they are composed of similar structural proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) protein and several non-structural proteins. Their members cause a variety of diseases in different vertebrate species [6, 8]. As of February 2020, seven human pathogenic coronaviruses are known: besides SARS-CoV[-1], SARS-CoV-2 and MERS-CoV, there are HCoV-HKU1, HCoV-OC43, HCoV-NL63 and HCoV-229E. While SARS-CoV[-1], SARS-CoV-2 and MERS-CoV can cause severe respiratory and systemic diseases, infections with the last four mentioned usually only lead to mild cold symptoms [3, 8].

After an infection with SARS-CoV-2, the virus accesses host cells via the protein ACE2 (angiotensin-converting enzyme) and causes the disease COVID-19. The severity of disease ranges from asymptomatic, mild (fever, cold, cough, tiredness, shortness of breath, and loss of smell), and severe to most severe forms with death [4, 5, 7]. Aging and several co-morbidities (e.g. diabetes mellitus, cardiovascular diseases, and chronic pulmonary diseases) are described as risk factors for severe progressive forms of COVID-19 [3, 12, 13].

Seroconversion occurs on different time points depending on the used method and the measured class of antibodies. Around 7 days after onset of symptoms, IgM antibodies against SARS-CoV-2 can be detected in the blood of majority of patients. The persistence of anti-SARS-CoV-2 antibodies in the blood decreases individually over a period of up to several weeks [9, 10, 11].

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 5001	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 5001	CONJ	Conjugate, ready-to-use	1 x 12.5 ml
K 5001	CTRL CUT-OFF	Cut-off control, ready-to-use	1 x 1 ml
K 5001	CTRL NEG	Negative control, ready-to-use	1 x 1 ml
K 5001	CTRL POS	Positive control, ready-to-use	1 x 1 ml
K 5001	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 110 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml
	FOL	Foil to cover the microtiter plate	3 x

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Microtiter plate thermoshaker at 37 °C (for example model Shake ID2 available at Immundiagnostik AG)
- A multi-channel dispenser or repeating dispenser
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. PREPARATION AND STORAGE OF REAGENTS

- **Bring all reagents to room temperature (18–30 °C) prior to use.**
- To run the assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary**

for each run. The kit can be used up to 4 times within the expiry date stated on the label.

- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** should be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. PREPARATION OF THE ASSAY

Sample storage

Freshly collected serum can be stored for 14 days at room temperature or for up to 4 weeks at 2–8 °C. Long-term storage is recommended at -20 °C. More than 3 freeze-thaw cycles should be avoided.

Diluted samples are **not stable** and **cannot be stored**.

Dilution of samples

Samples are diluted **1:101 in sample dilution buffer**. For example:

- **10 µl** sample + **1000 µl** sample dilution buffer, mix well = **1:101**

For analysis, pipet **100 µl diluted sample** per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA serves for the determination of IgM antibodies against the spike protein (S1) of SARS-CoV-2. Diluted samples are added to a microtiter plate coated with a specific antigen. By adding the peroxidase conjugate (peroxidase labelled detection antibody), the antibodies against SARS-CoV-2 in the sample are marked. They are detected via the peroxidase conjugate with the peroxidase converting the substrate TMB to a blue product. The enzymatic reaction is stopped by adding an acidic solu-

tion. The samples convert from blue to yellow. The colour change should be measured in a photometer at 450 nm. The interpretation is made using the cut-off control.

Test procedure

Bring all reagents and samples to room temperature (18–30 °C) and mix well.

Mark the positions of controls/samples on a protocol sheet.

Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered with the foil included in the kit together with the desiccant bag in the re-closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details, please contact your supplier or Immundiagnostik AG.

We recommend carrying out the tests in duplicates.

1.	Pipet each **100 µl of controls and diluted samples** into the wells of the microtiter plate.
2.	Cover the strips and incubate for **1 hour shaking (900 rpm)*** on a horizontal shaker at 37 °C.
3.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
4.	Add **100 µl conjugate** (CONJ) into each well.
5.	Cover the strips and incubate for **1 hour shaking (900 rpm)*** on a horizontal shaker at 37 °C.
6.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
7.	Add **100 µl substrate** (SUB) into each well.
8.	Incubate for **10–15 minutes** at room temperature (18–30 °C) until a sufficiently large difference in colour occurs**.
9.	Add **100 µl stop solution** (STOP) into each well and mix shortly by using the **shake function (900 rpm)*** of the microplate reader.

10.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference.

* We recommend shaking the strips with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

Cut-off = OD cut-off control x 1.2

Samples which have a higher average optical density (OD) than the **cut-off** are positive.

Samples which have a lower average optical density (OD) than the **cut-off** are negative.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a manual control of the paired values should be made.

9. LIMITATIONS

Samples which cannot be clearly interpreted (e. g. because of high coefficients of variation of replicates) should be measured again.

Negative IgM results do not rule out an infection with SARS-CoV-2. The serum or plasma samples may be collected at a very early stage of infection when the body has not yet produced IgM antibodies. They are detectable earliest 2-3 days after the start of an infection. A certain time after virus infection, IgM signal starts to decrease and negative results are expected.

This test serves as indirect virus detection method and does not replace a direct virus detection.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

11. PERFORMANCE CHARACTERISTICS

Clinical specificity and sensitivity

Sensitivity; n = 20

For determination of clinical sensitivity, 20 samples from patients with previous RT-PCR confirmed SARS-CoV-2 infection were tested. Blood samples, used for determination of sensitivity were collected at later stages of infection.

	Number of patients with positive RT-PCR
total	20
Positive IgM antibody detection	19
Negative IgM antibody detection	1

These results in a sensitivity of 95 % for the IDK® anti-SARS-CoV-2 IgM ELISA.

Specificity; n = 762

For determination of clinical specificity, 762 serum samples from blood donors were tested. All samples were collected in 2017 and 2018.

	Number of blood donors
total	762
Positive IgM antibody detection	18
Negative IgM antibody detection	744

The resulting specificity for the IDK® anti-SARS-CoV-2 IgM ELISA was 97.6 %.

Cross-reactivity

Specificity data were confirmed using serum samples, which have been tested positive for antibodies against different viruses (n=47). These data showed a specificity of 96 % for the IDK® anti-SARS-COV-2 IgM ELISA.

Virus	**n**	**positive [%]**	**negative [%]**
Adenovirus	5	20	80
Epstein-Barr virus	5	0	100
Influenza A/B	5	0	100

Virus	n	positive [%]	negative [%]
HCoV-229E	5	0	100
HCoV-HKU1	7	0	100
HCoV-NL63	12	8	92
HCoV-OC43	8	0	100
Total	**47**	**4**	**96**

Accuracy – Precision

Repeatability (Intra-Assay)

The repeatability was assessed with 2 serum samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [OD]	CV [%]
1 (n=90)	2.855	3.49 %
2 (n=90)	0.435	4.30 %

Reproducibility (Inter-Assay)

The reproducibility was assessed with 1 serum sample under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [OD]	CV [%]
1 (n=20)	2.071	13.1
2 (n=20)	0.362	16.2

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. LITERATURE

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Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet