COVELISA®
COVID-19 IgM ELISA

Instructions for use

COVELISA®COVID-19 IgM ELISA Σ **96 determinations** REF **LIO-COV19-IgM**

The COVELISA®COVID-19 IgM ELISA is an in vitro semi-quantitative detection test of human antibodies of the immunoglobulin class IgG directed against SARS-CoV-2 from human serum or plasma to aid the diagnosis of SARS-CoV-2 infection and to complement the direct detection of the pathogen.

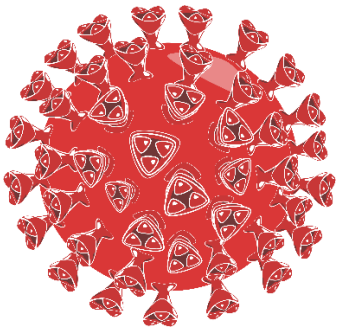
Serology can also be used to collect epidemiological data. The product is intended for use as an IVD, but can also be used for research purposes.

IVD Only for in-vitro diagnostic use!

CE

Manufacturer:
LIONEX GmbH
Salzdahlumer Strasse 196, Geb. 1A
38126 Braunschweig, GERMANY
Phone: +49 (0) 531 - 260 12 66
Fax: +49 (0) 531 - 618 06 54
Web: www.lionex.de

www.lionex.de
Please contact sales@lionex.de



LIONEX Diagnostics and Therapeutics

Contents

Intended use

The **COVELISA®COVID-19 IgM ELISA** is an in-vitro semi-quantitative detection test of human antibodies of the immunoglobulin class IgM directed against SARS-CoV-2 from human serum or plasma to aid the diagnosis of SARS-CoV-2 infection and to complement the direct detection of the pathogen.

Serology can also be used to collect epidemiological data. The product is intended for use as an IVD, but can also be used for research purposes.

Introduction / Field of application

Severve Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, formerly 2019-nCoV) is an enveloped, non-segmented positive-sense RNA virus. This virus causes Coronavirus Disease 2019 (COVID-19), which is highly contagious in humans.

SARS-CoV-2 has structural proteins such as spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) is a transmembrane protein consisting of the S1 subunit and the S2 subunit. The S1 subunit contains a receptor binding domain (RBD) responsible for the recognition of the cell surface receptor. The RBD domain of the SARS-CoV-2 S protein was found to interact strongly with the human ACE2 receptor.

The virus is transmitted by droplet infection (mainly coughing, sneezing but also smear infection) and causes infection of human respiratory epithelial cells. The symptoms of SARS-CoV-2 infection are fever, cough, difficulty breathing and exhaustion. Especially elderly and chronically ill people can develop a severe acute respiratory syndrome (SARS) with consequences of death (about 3% of cases).

The immune response causes immunoglobulin antibodies such as IgA, IgM and IgG to appear in the blood. IgA and IgM antibodies are an early indicator of infection (approx. 1 - 2 weeks after disease onset), while IgG antibodies are an important indicator of recent and past infection (approx. 2 - 4 weeks after disease onset).

The antibody determination does not replace the direct detection by PCR. In the delayed early phase, however, in some cases the detection of IgM or IgA antibodies can increase the sensitivity of the detection. In case of an acute infection, the typical "positive" constellation would be IgM/IgA positive and IgG (still) negative.

However, if the IgM or IgA result is positive, it should be noted that about 12 - 14% of cases can be false positive. If the result is positive, a further examination 10 - 14 days later can confirm a seroconversion to IgG.

For the detection of a past infection with SARS-CoV-2 (convalescence phase, already recovered), IgG is of particular importance.

According to previous studies, antibodies appear at the earliest 5 - 8 days after virus transmission. In the first 7 days after the onset of symptoms, the rate of positive results for IgA or IgM is about 50%, for IgG even lower, but increases significantly thereafter. Patients with symptoms show seropositivity for IgG in about 98% of cases 14 - 21 days after the onset of the disease. It is not yet certain whether these results can be transferred to asymptomatic disease progression.

Principle of the test

The **COVELISA®COVID-19 IgM ELISA** is a microtiter plate based enzyme test for the semi-quantitative detection of IgM antibodies against SARS-CoV-2 in human serum and plasma.

The **COVELISA®COVID-19 IgM ELISA** test is based on the principle of the enzyme immunoassay (EIA). Specific recombinant antigens S1 is bound on the surface of the microtiter plates. Diluted patient samples are pipetted into the wells simultaneously with the controls. During a 60-minute incubation period at room temperature (RT), specific antibodies contained in the samples bind to the immobilized antigen. A wash step is then performed to remove unbound material. Now the ready-to-use conjugate (peroxidase-coupled anti-human antibody) is pipetted into the wells. During a second incubation (30 minutes / room temperature (RT)) the conjugate binds to the already immobilized specific antibodies. After another washing step, the substrate for the peroxidase is added (TMB). The plate is then incubated for 20 minutes at room temperature (RT). During this time the peroxidase activity causes a color change from colorless to blue. The intensity of the reaction depends on the number of specific antibodies in the samples. The colour development is interrupted after 20 minutes by the addition of a stop solution. The colour changes from blue to yellow. The concentration of antibodies in the sample is directly proportional to the intensity of the colour. The optical density is measured spectrophotometrically on the ELISA reader at 450 nm.

Supplied materials

Description	Colour	Symbol	Formate
COVELISA®COVID-19 IgM ELISA		Σ	96 determinations
		REF	**LIO-COV19-IgM**
Mikrotiter plate (ready-to-use), coated with recombinant SARS-Cov-2 antigen, sealed in a resealable aluminium pouch with desiccant, 12 x 8 breakable wells		MTP	12 x 8 (96)
Wash buffer (10x concentrate)	■	WPT	100 mL
Sample diluent (ready-to-use)	□	TP1	50 mL
Negative control (ready-to-use), contains low concentration of relevant antibodies against SARS-CoV-2	■	NEG	2 mL
Calibrator (ready-to-use), contains defined concentration of relevant antibodies against SARS-CoV-2	■	CAL	2 mL
Positive control (ready-to-use), contains high concentrations of relevant antibodies against SARS-CoV-2	■	POS	2 mL
Conjugate Solution (ready-to-use), contains anti-human IgM conjugate (Fc5µ)	■	CON	14 mL
Substrate Solution* (ready-to-use), contains TMB	■	TMB	14 mL
Stop Solution* (ready-to-use), contains H_2SO_4	■	STO	14 mL
Instructions for use			1 x

* Refer to section "*Warnings and Precautions*".

Materials needed but not provided

- Tubes for sample collection and dilution (optionally, e.g. sterile 1.5 mL tube)
- Beakers, flasks and/or graduated cylinders necessary for 1x wash buffer preparation
- Deionized or destilled water
- Calibrated variable pipets for a volume of 10 µL to 1000 µL with disposable tips
- Microtiter Plate-Washer (recommended) or calibrated multichannel pipets for a volume of 100 µL to 300 µL with disposable tips (optionally)
- Timer
- Microtiter Plate-Reader (450 nm, optional 620 nm)

Test procedure time

The required time to perform the **COVELISA®COVID-19 IgM ELISA** is approx. 150 minutes.

Warnings and Precautions

For in-vitro diagnostic use! Not for personal use!

- Follow the instructions of the test procedure and interpretation of results carefully!
- In accordance with Good Laboratory Practice (GLP), all laboratory devices employed should be regularly checked and calibrated for the accuracy and precision.
- Some reagents contain preservatives in concentrations not subject to declaration. Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Do not work without wearing protective clothing (disposable gloves, safety glasses and lab coat)! Avoid the contact of kit reagents with skin, eye or mucosa.
- All kit components should be considered as infectious. Decontaminate and dispose the kit componetents (microtiter plate and liquids) and samples according to local regulations, e.g. by autoclaving or using a disinfectant solution.
- Use all reagents within the expiry period (printed on the labels).
- Do not use reagents from different kit lots or batch codes and do not mix reagents of different kit lots or batch codes.
- Bring all test components to room temperature (preferably 15 - 30°C) and invert liquid test components gently before use. Return test components immediately to 2 - 8°C after usage. The test is sensitive to temperatures above 30°C.
- Body fluids other than human serum or plasma are not validated and can yield incorrect results!
- Avoid contamination of the reagents. Do not use the same container for several samples! Avoid contamination of reagents by changing pipette tips. Close the bottles tightly again immediately after removing reagents.
- Lipemic, hemolytic, icteric or bacterially contaminated samples should not be used.
- We recommend the use of double determinations for measurements.
- Avoid repeated freezing and thawing of the samples because it could lead to denaturation of the antibodies.
- Before pipetting, mix all reagents thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. Pipet with constant intervals, so that all wells of the microtiter plate have the same conditions.

- Protect the **TMB** Substrate Solution from direct sunlight.

For more information, please request the **Material Safety Data Sheets (MSDS)** via E-mail to sales@lionex.de.

ATTENTION:
Handle human serum and plasma as potentially infectious. All kit components should be considered as infectious agents. Decontaminate and dispose remaining kit reagents and human samples in accordance with federal, state and local regulations, e.g. by autoclaving or using a disinfecting solution.

Hazard and Precautionary Statements

TMB **Substrate Solution**
3,3', 5,5'-Tetramethylbenzidin (TMB) contains: N-Methyl-2-pyrrolidon. Danger! May damage fertility or the unborn child (H360D).
Dispose the kit components to an approved waste disposal plant.
If exposition occurred: Get medical advice and obtain special instructions before use. Wear protective gloves and lab coat (eye protection, face protection).

STO **Stop Solution**
Contains: Sulfuric acid. Danger! Causes severe skin burns and eye damage (H314). May be corrosive to metal (H290).
Dispose the kit components to an approved waste disposal plant.
If contact with eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if they are present and easy to remove. Continue rinsing.
If contact with skin: Remove immediately all contaminated clothing. Rinse skin with water. Immediately call a poison center or doctor. Wear protective gloves and lab coat (eye protection, face protection).

Sample collection and preparation

The **COVELISA®COVID-19 IgM ELISA** test is suitable for the detection of IgM antibodies against SARS-CoV-2 in human serum and plasma. The test works best with fresh samples.

Collection of whole blood from the vein: Collect the sample under standard laboratory conditions (aseptically and in such a way that haemolysis is avoided).

Serum or plasma: Separate from red blood cells as soon as possible (e.g. by centrifugation). If the test cannot be performed immediately after sample collection, samples may be stored at 2 - 8°C for up to 14 days. Serum and plasma can also be stored at temperatures below -20°C. Frozen samples must be thawed and mixed thoroughly before testing. Avoid repeated thawing and freezing of samples!

Dilute the samples 1:50 in **TP1** Sample diluent (e.g. 490 µL buffer + 10 µL sample)! Diluted samples should be used within one day for the test procedure.

Preparation of reagents

Bring all test components to room temperature (preferably 15 - 30°C, approx. 30 minutes) and invert liquid test components gently before use.

WPT Wash buffer (10x concentrate):

If crystals precipitate during the cold storage, the concentrate should be warmed up at 37°C for 15 minutes. Before use, dilute 1:10 with distilled water (e.g. 100 mL buffer + 900 mL ddH_2O).

Stability and storage conditions

- **Store** all test components **at 2 - 8°C**.
- **DO NOT FREEZE** the test components.
- **DO NOT EXPOSE** the test components to temperatures **above 30°C**.
- Protect the **TMB** Substrate Solution from direct sunlight.

The **MTP** microtiter plate is sensitive to moisture. After first opening of microtiter plate the stability is given up until expiry date, if the remaining microtiter plate stripes will be stored at 2 - 8°C in an aluminium bag (closed by zipper) with desiccant.

After dilution of the **WPT** Wash Buffer (10x concentrate) the diluted solution (1x wash buffer) is stable for 1 month at 2 - 8°C. For instructions on how to prepare the 1x wash buffer, please see section "*Preparation of reagents*".

Unopened kit components are stable until the expiry date. The expiry date is printed on the labels of each test component and on the outer packaging. Do not use if test components are damaged or open (e.g. no screw cap, aluminium bag damaged). After first opening of liquid components the stability is given until the expiry date, if the bottles are tightly closed and protected from contamination after every usage.

Test procedure

1. Test time is about 150 minutes. Refer also to section "*Abbreviated Test procedure*"! Test preparation: Refer to section "*Preparation of Reagents*": Bring all components of the kit to room temperature (15 - 30°C, approx. 30 minutes) and invert liquid test components gently before use.

   WPT Wash Buffer: dilute 10x concentrate 1:10 with deionized or distilled water (e.g. 100 mL buffer + 900 mL ddH_2O).

   Serum and plasma samples should be thoroughly mixed before use. Dilute samples 1:50 with **TP1** Sample diluent (e.g. 490 µL buffer + 10 µL sample).

2. Take the **MTP** microtiter plate out of the aluminium bag and place the required microtiter strips in the holder provided. Put the unused microtiter strips back into the aluminium bag.

3. Pipet 100 µL of the diluted samples and the undiluted **POS**, **NEG** controls and **CAL** Calibrator as well as **TP1** Sample diluent as blank value per well. **POS**, **NEG**, **CAL** and **TP1** . We recommend to measure the controls in duplicates.

   INCUBATION DURATION: 60 minutes at RT (15 - 30°C)

4. Washing step: Remove the liquid from the wells (aspirate using an automatic washer) and wash 6 times with 300 µL/well of diluted Wash Buffer (1x).

5. Pipet 100 µL of the **CON** Conjugate Solution into the wells.

   INCUBATION DURATION: 30 minutes at RT (15 - 30°C)

6. Washing step: Remove the liquid from the wells (aspirate using an automatic washer) and wash 6 times with 300 µL/well of diluted Wash Buffer (1x).

7. Pipet 100 µL of **TMB** Substrate Solution into the wells.

   INCUBATION DURATION: 20 minutes at RT (15 - 30°C) in the dark

8. Pipet 100 µL of **STO** Stop Solution into the wells to stop the substrate reaction (no washing!).

9. Measurement on the ELISA Reader: Measure the absorbance (OD) at 450 nm (optional reference wavelength: 620 nm). The color is stable for at least 60 minutes.

Interpretation of test results

The ready-to-use controls and calibrator contain different concentrations of relevant antibodies to SARS-CoV-2:

NEG	Negative control contains low concentration of relevant antibodies against SARS-CoV-2
CAL	Calibrator contains defined concentration of relevant antibodies against SARS-CoV-2
POS	Positive control contains high concentration of relevant antibodies against SARS-CoV-2

Calculate the OD mean value for **each sample** and each control/calibrator from the two OD values of the double determination, if measured in duplicates. The difference between the two single OD values must not be greater than 10%, otherwise the test must be repeated for this sample(s).

Subtract the OD blank value from each calculated OD mean value.

OD mean value		**OD blank value**
POS Positive control, **CAL** Calibrator, **NEG** Negative control, Diluted samples	–	**TP1** Sample diluent

The calculated values of each sample can be compared to the values measured for the controls/calibrator. To reduce variation (from day to day, etc.) the measured values should be normalized (NV). For this, divide all calculated values for the samples by the value of **CAL** Calibrator:

$$\textbf{Normalised value (NV)} = \frac{\textbf{OD sample - blank}}{\textbf{OD CAL - blank}}$$

The test result can be **NEGATIVE**, **BORDERLINE** or **POSITIVE**.

The normalied values (NV) for the test were determined by measuring various serum and plasma samples from COVID-19 patients and healthy individuals.

Based on our own results we have determined the following normalied values (NV) for the **COVELISA®COVID-19 IgM ELISA**:

NV (Normal. value)	Test result
< 0.4	**NEGATIVE**
0.4 – 0.5	**BORDERLINE**
> 0.5	**POSITIVE**

If the results are invalid (QC criteria not fulfilled, refer also to section "*Quality control of the test*"), refer to section "*Troubleshooting Guide*" to identify possible causes.

If a result is "borderline", the result is not clear. In this case take another sample from the same patient after 1 - 2 weeks and repeat the measurement to investigate possible changes in titer.

The diagnosis "COVID-19" should be made on the basis of the overall clinical analysis and, if possible, taking all test results into account! For a reliable diagnosis, we recommend to include all available clinical data (PCR, disease symptoms etc.).

Quality control of the test

The **COVELISA®COVID-19 IgM ELISA** contains internal controls. After successful completion of the test, a blue coloration will appear in the wells into which the **POS** Positive control was pipetted. After addition of the **STO** Stop Solution the color changes from blue to yellow. This color development serves as a positive procedural control. It confirms sufficient sample volume and correct test procedure. A clear background in the wells into which only **TP1** Sample diluent (blank value) or the **NEG** Negative Control was pipetted is the internal negative test control.

The absorbance (OD) of the Calibrator and the Positive and Negative Controls must be within the tolerances specified for the test lot. A certificate of analysis (COA) with the corresponding data is attached. If these control requirements are not reached, the test results may be inaccurate (invalid) and the test should be repeated.

INVALID: If the criteria above are not fulfilled, the analysis of the test results will be affected, and the test is invalid. The colour of the controls should change from light blue to dark blue after the pen-ultimate step (addition of **TMB** Substrate Solution, after 20 minute-incubation). If no blue coloration is visible in the wells containing the controls, the test result is invalid. If a background coloration appears in the wells with the blank value, the result may be invalid.

Insufficient sample volume or incorrect handling of the test procedure are the most likely reasons for not reaching the required QC criteria of test performance. If the OD value of **TP1** Blank is > 0.15, the washing procedure should be improved. Check again the instructions of sample preparation and test procedure and repeat the test with a new microtiter strip device. If the problem persists, contact the manufacturer or your local distributor.

Limitations

Follow the instructions of the test procedure and interpretation of results carefully! Insufficient sample volume or incorrect handling of the test procedure are the most likely reasons for not reaching the required QC criteria of test performance (see section "*Quality control of test*").

The IgM antibodies are ONLY produced by the immune system in very early stages of the disease, therefore specific IgM antibodies are only detectable in a limited percentage of all samples from patients! The **COVELISA®COVID-19 IgM ELISA** was developed exclusively for the detection of early stages of the disease. This kit complements the "COVID-19 ELISA – Human IgG" kit. For a final diagnosis and prevalence studies, the samples should therefore also be tested for the presence of the antibody class IgG.

A POSITIVE test result suggests that an infection with SARS-CoV-2 is likely. A NEGATIVE result indicates that infection is unlikely. Note that questionable results (BORDERLINE) require further confirmation. If the result is BORDERLINE, another sample should be taken from the same patient after 2 - 4 weeks and checked again.

The antibody determination does not replace the direct detection by PCR. It is important to note that a positive IgM result against SARS-CoV-2 indicates that an infection has taken place, but this does not necessarily mean that immunity (i.e. protection against infection) is assured. The long-term studies necessary for this statement cannot yet exist.

Cross-reactions of antibodies within the genus *Betacoronavirus* can occur.

It is advised to consider the results from test in combination with each individual´s clinical status, results of other diagnostic tests and the background epidemiological information.

If a patient sample has tested as positive, further confirmatory tests should be performed (e.g. PCR, clinical symptoms). For a final diagnosis, include all available information on a particular patient.

Similarly, a negative test result does not exclude a possible infection.

For meaningful serological results, we recommend that two samples from the same patient should be tested, the first from the acute phase (week 1 of the disease) and a second sample additionally from the convalescence phase (3 to 4 weeks after the disease).

The test has been developed for the detection of human SARS-CoV-2 in serum or plasma. For detecting IgM against SARS-CoV-2 in body fluids other than human serum or plasma the test has not been validated and can yield incorrect results.

While harvesting human serum or plasma avoid contamination by red blood cells. If necessary, separate the red blood cells from serum or plasma by centrifugation. Avoid bacterial contamination of the samples!

Samples with absorbance (OD) values exceeding the positive control should be reanalyzed at higher dilutions. For further analysis the samples can be diluted e.g. 1:100 with **TP1** Sample diluent (10 µL sample in 990 µL buffer).

As with all diagnostic tests, a definitive clinical diagnosis should not be based solely on the result of one test, but should be made by a physician based on an evaluation of all clinical and laboratory findings.

In order to get a more accurate picture of the status of the disease, we recommend measuring the same sample by IgM- and IgG ELISA. If IgM only or IgM and IgG appears, the patient is fighting the infection. If IgG only appears, the patient has probably recovered.

Warning: Samples from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or decreased values when tested with test kits such as SARS-CoV-2 IgM using mouse monoclonal antibodies.

Heterophilic antibodies in human serum may react with reagent immunoglobulins and interfere with in-vitro immunoassays. Patients routinely exposed to animals or animal serum products may be susceptible to this interference and abnormal values may occur.

Rheumatoid factor (RF) in human serum may react with reagent immunoglobulins and interfere with in-vitro immunoassays.

Interfering substances

Analytical specificity is determined by measuring potential interfering substances. Substances used for patient treatment, substances which may be ingested by the patient and substances encountered in specific specimens types are taken into account. The following substances tested in the concentrations mentioned below did not interfere test results:

Bilirubin	0.4 mg/mL
Haemoglobin	10 mg/mL
Triglyceride	20 mg/mL

To be on the safe side we recommend to exclude haemolysed, lipemic and icteric samples from testing.

Performance Characteristics

Measuring range

Limit of Quantitation (LoQ) and Limit of Blank (LoB) of the **COVELISA®COVID-19 IgM ELISA** are determined by calculating from blank values (22 repetitions, different batches and operators on different days, values in OD). The following values are calculated from the data measured:

Limit of Quantitation (LoQ):	0.174
Limit of Blank (LoB):	0.061

Because the test is semi-quantitative, the calculation of the detection limit (LoD) was not applicable.

Analytical sensitivity

The determination of the analytical sensitivity was not applicable because the test is semi-quantitative and the cut-off is defined far above the detection limit. Furthermore, no standards were available.

Analytical specificity

Interferences and cross-reactivity are evaluated by taking into account the available clinical data (from various similar test manuals and publications). Potential interfering substances were added to the samples to check the analytical specificity. Refer to section "*Interferences*".

Precision

In order to estimate the reproducibility of the measurements, intra- and inter-assay variations as well as inter-operator variations and batch-to-batch variations were determined by measuring samples of different reactivity by **COVELISA®COVID-19 IgM ELISA**.

Inter-assay variation was determined by repeated measurements of 3 samples of different reactivity by 3 different batches on 10 different days (negative, weakly positive, positive sample). The inter-assay variation (CV (%))was 6.198% for positive sample 1 and 12.834% for positive sample 2. For the negative sample (= zero sample) the variation was 29.858%.

The **variation in the intra-assay** or within the run was determined by repeated measurements of samples with different reactivity (3 samples with different reactivity: negative, weakly positive, positive sample; 20 repetitions). The intra-assay variation was between 7.860% - 13.714% (CV (%)).

The **batch-to-batch variation** was determined by measuring 12 samples of different reactivity with 3 different batches. The variation (CV (%)) was 6.284% - 19.665%. As expected, a higher coefficient of variation than 10% was observed in the samples with low concentrations. At low values the variation is known to be greater than at higher values. The average coefficient of variation calculated for all samples was 16.32%.

To determine the **inter-operator variation**, 13 samples with different reactivity were measured by 2 different operators on different days. The inter-operator variations were between 1.828% and 66.804%. For the positive samples (COVID-19) the coefficient of variation was less than 10% (1.828% – 9.115%). In the negative samples the coefficient of variation (CV (%)) was between 4.009% – 66.804%. The variation at low concentrations is usually greater than at higher concentrations. The mean variation was acceptable at 19.102%.

High-Dose-Hook-Effect: Human serum was spiked with high concentrations of polyclonal anti-IgG antibodies against S1 and measured with the **COVELISA®COVID-19 IgM ELISA**. No high-dose-hook effect was observed at high concentrations of the antibody (diluted 1:100).

Diagnostic sensitivity and specificity

96 negative plasma or serum samples were measured by **COVELISA®COVID-19 IgM ELISA** (samples from patients with other lung diseases, from clinical laboratories or healthy individuals who have never been exposed to SARS-CoV-2). Furthermore, 16 positive samples from RT-PCR positive COVID-19 patients (taken 6 – more than 54 days after the first symptoms appeard) were tested. A total of 113 samples were used to calculate the agreement of the **COVELISA®COVID-19 IgM ELISA** with the reference method.

The sensitivity for the measured panel was 62.5% with a specificity of 97.9% (confidence interval (CI): 92.7 – 99.7% for the cut-off = 0.5 (NV)).

The longer the time span after the appearance of the first symptoms, the higher the number of positive results:

Duration of symptoms	Sensitivity	Number of samples
Day 10 or more	76.92 %	13
Day 14 or more	90.00 %	10

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems which could occur. If the problem persists, contact the manufacturer or your local distributor (see back cover).

Nonspecific colour development or high background	
Possible reason	**What to do**
Incomplete washing of microtiter plate	Wash the plate at least 6 times with 300 µL/well of diluted Wash Buffer (1x).
The expiration date of kit components has passed.	Use the kit components before expiry date. Pay attention to the expiry of opened **MTP** microtiter plate mentioned in "*Stability and storage conditions*".
Incubation temperature is too high	Incubation of the ELISA should be done at room temperature (15 - 30°C).
Mixing or dilution forgotten	Ensure each step is done. Check again the instructions of sample preparation and test procedure and repeat the test with a new microtiter strip device.
Cross-contamination	Take care when pipetting the samples and solutions. If **TMB** Substrate Solution shows blue dye (before use), discard the solution. Pay attention to use clean reagent reservoirs.
Low optical density of controls, calibrator	
Possible reason	**What to do**
Pipetting mistake	Pipets should be calibrated and be used according to manufacturer`s instructions.
The expiration date of kit components has passed.	Use the kit components before expiry date. Pay attention to the expiry of opened **MTP** microtiter plate mentioned in "*Stability and storage conditions*".
Incubation temperature is too low	Incubation of the ELISA should be done at room temperature (15 - 30°C).
Incubation time is too short	Incubation time of the **NEG**, **POS** controls, **CAL** calibrator, **TP1** Blank and diluted samples is 1 hour (60 minutes). Incubation time of **CON** Conjugate Solution is 30 minutes. The incubation time of **TMB** Substrate Solution is 20 minutes.
Reagents are too cold	All reagents should be warmed up to room temperature (15 - 30°C).
Incorrect plate reader filter is used	Plate should be read out within 60 minutes after addition of **STO** Stop solution at 450 nm (optional reference wavelength: 620 nm).
Duplicate variability	
Possible reason	**What to do**
Incomplete washing of microtiter plate	Wash the plate at least 6 times with 300 µL/well of diluted Wash Buffer (1x).
Insufficient mixing	Ensure dilutions and mixing of the samples are prepared according to instructions for use.
Incorrect continuity of pipetting technique or interruption during test procedure	Pipetting of **NEG**, **POS** controls, **CAL** calibrator, **TP1** Blank and diluted samples should be performed continuously. All reagents should be prepared before starting the assay procedure.

References

Daniel Wrapp, Nianshuang Wang, Kizzmekia S. Corbett, Jory A. Goldsmith, Ching-Lin Hsieh, Olubukola Abiona, Barney S. Graham and Jason S. McLellan. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. originally published online February 19, 2020 DOI: 10.1126/science.abb2507 (6483), 1260-1263. 367 Science

Chan JFK, Lau SKP, To KKW, Cheng VCC, Woo PCY, Yuen K-Y. Middle East respiratoy syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disearse. Clin Microbiol Rev. 2015; doi:10.1128/CMR.00102-14

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3): Pii=2000045

Gallagher and Buchmeier (2001). Coronavirus spike proteins in viral entry and pathogenesis. Virology. 279(2):371-4.

Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCov. Viruses 2020, 12(2), 135

Hoffmann et al., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a ClinicallyProven Protease Inhibitor, Cell (2020), https://doi.org/10.1016/j.cell.2020.02.052.

Juanjuan Zhao et al. Antibody Responses to SARS-CoV-2 in Patients of Novel Coronavirus Disease 2019" Clin Infect Dis . 2020; doi: 10.1093/cid/ciaa344

Li F. (2016). Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol. 3(1):237-261.

Okba NMA, Muller MA, liW, Wang C, GeurtsvanKessel CH, Corman VM, et al. SARS-CoV-2 specific antibody responses in COVID-19 patients. MedRxiv preprint. 2020, doi: 10.1101/2020.03.18.20038059

Okba NMA et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerg Infect Dis. 2020 Apr 8;26. doi: 10.3201/eid2607.200841.

Su et al. (2016). Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol. 2016 Jun; 24(6):490-502.

Wang G, Jin X. The progress of 2019 Novel Coronavirus (2019-nCoV) event in China. J Med Virol. Doi: 10. 1002/jmv.25705

Weiss SR, Leibowitz JL. Novel coronavirus (2019-nCoV), World health Organisation (WHO), 2020. Coronavirus pathogenesis. Adv Virus Res 2011;81:85-164. PMID:22094080 DOI:10.1016/B978-0-12-385885-6.00009-2

World Health Organization (WHO). WHO Statement Regarding Cluster of Pneumonia Cases in Wuhan, China. Beijing: WHO; 9 Jan 2020. [Accessed 26 Jan 2020]. https://www.who.int/china/news/detail/09-01-2020-who-statement-regarding-cluster-of-pneumonia-cases-in-wuhan-china

World Health Organization (WHO). Coronovirus. https://www.who.int/health-topics/coronavirus

WHO: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases, Interim giudance, 17 January 2020

WHO (2020). Novel Coronavirus(2019-nCoV) Situation Report 23. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200212-sitrep-23-ncov.pdf?sfvrsn=41e9fb78_4.

Xiao SY, Wu Y, Liu H. Evolving status of the 2019 novel coronavirus Infection: proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020; 1-4

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020). A pneumonia outbreak associated with anew coronavirus of probable bat origin. Nature. https://doi.org/10.1038/s41586-020-2012-7.

Symbols

IVD	For in vitro diagnostic use
CE	Compliant with IVD Directive 98/79/EG
GTIN	Global Trade Item Number
REF	Catalogue Number
LOT	Batch code
	Manufacturer
Σ	Contains sufficient amount for <n> tests
	Consumables: use by … (expiry date)
2°C 8°C	Store at 2 - 8°C (temperature limitation)
2°C 30°C	Store at 2 - 30°C (temperature limitation)
	Please consult instructions for use
	Do not reuse
	Do not use if damaged
	Protect from moisture
	Keep away from sunlight

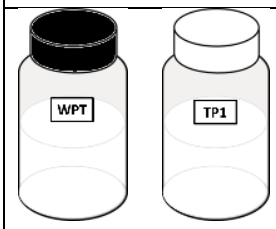
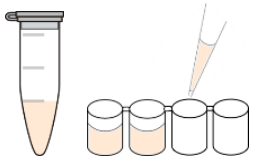
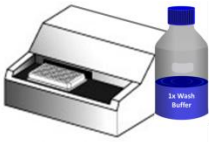
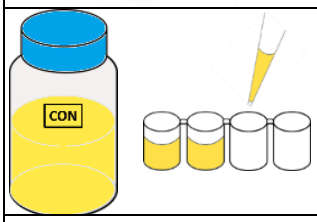
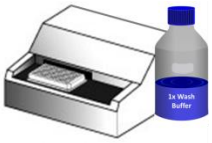
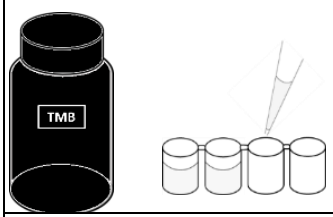
MTP	Microtiter plate
WPT	Wash Buffer, 10x concentrate
TP1	Sample diluent
CON	Conjugate Solution
NEG	Negative control
CAL	Calibrator
POS	Positive control
TMB	Substrate Solution
STO	Stop Solution

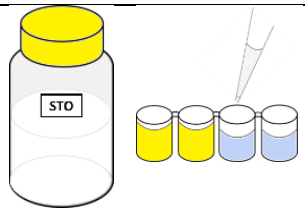
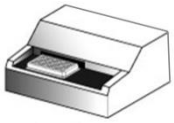
Contact Information

For more information and technical assistance, please contact

sales@lionex.de
or call + 49 (0) 531 - 260 12 66
or visit our homepage www.lionex.de

Abbreviated Test procedure

	**ATTENTION! Please read the instructions for use carefully!**
	Bring all components of the kit to room temperature (15 - 30°C, approx. 30 minutes) and invert liquid test components gently before use.
	**WPT** Wash Buffer: dilute 10x concentrate 1:10 with deionized or distilled water (e.g. 100 mL buffer + 900 mL ddH2O). Serum and plasma samples should be thoroughly mixed before use. Dilute samples 1:50 with **TP1** Sample diluent (e.g. 490 µL buffer + 10 µL sample).
	Take the **MTP** microtiter plate out of the aluminium bag and place the required microtiter strips in the holder provided. Put the unused microtiter strips back into the aluminium bag.
	Pipet 100 µL of the diluted sample and the undiluted **POS**, **NEG** controls, **CAL** calibrator as well as **TP1** Sample diluent as blank value per well. We recommend to measure samples, controls, calibrator and blanks in duplicates.
INCUBATION DURATION: 60 minutes at RT (15 - 30°C)	
	Washing step: Remove the liquid from the wells (aspirate using an automatic washer) and wash 6 times with 300 µL/well of diluted Wash Buffer (1x).
	Pipet 100 µL of the **CON** Conjugate Solution into the wells.
INCUBATION DURATION: 30 minutes at RT (15 - 30°C)	
	Washing step: Remove the liquid from the wells (aspirate using an automatic washer) and wash 6 times with 300 µL/well of diluted Wash Buffer (1x).
	Pipet 100 µL of **TMB** Substrate Solution into the wells. **CAUTION/DANGER** 
INCUBATION DURATION: 20 minutes at RT (15 - 30°C) in the dark	

	Pipet 100 µL of **STO** Stop Solution into the wells to stop the substrate reaction (no washing!). **CAUTION/DANGER** 
	Measurement on the ELISA Reader: Measure the absorbance (OD) at 450 nm (optional reference wavelength: 620 nm). The color is stable for at least 60 minutes.

Trademarks:

LIONEX®

Limited License Agreement for COVELISA®COVID-19 IgM ELISA.

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used only with the instructions provided in the kit and the kit components. LIONEX GmbH grants no license under any of contained the enclosed components of this kit any components which are not included within this kit (except as described in the instruction and the kit) or of intellectual ability.

2. Other than expressly licenses, LIONEX GmbH gives no warranty the kit and/or its use(s) so not break the rules and fringe the rights of third-parties.

3. The kit and its components are licensed for one-time use and it`s not allowed to reuse the kit components. To process different or resold unless otherwise defined by LIONEX GmbH.

Manufacturer:

LIONEX GmbH

Salzdahlumer Strasse 196, Geb. 1A
38126 Braunschweig, GERMANY
Phone: +49 (0) 531 - 260 12 66
Fax: +49 (0) 531 - 618 06 54
Web: www.lionex.de

Distribution:

Address label
of distributor