# ELISA-VIDITEST anti- SARS-CoV-2 (NP) IgM

REF **ODZ-471** Σ 96

**Instruction manual**

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## 1. TITLE:

ELISA-VIDITEST anti-SARS-CoV-2 (NP) IgM – ELISA kit for the detection of IgM antibodies to nucleocapsid protein (NP) of coronavirus SARS-CoV-2, causing the COVID-19 disease in human serum and plasma.

## 2. INTRODUCTION:

After infection of a human body with the SARS-CoV-2 virus, the antibodies to this virus appear in the patient's blood. Most persons infected with SARS-CoV-2 display an antibody response between day 10 and day 21 after infection. Detection in mild cases can take longer time (four weeks or more) and in a small number of cases antibodies (i.e., IgM, IgG) are not detected at all (at least during the studies' time scale). Based on the currently available data, the IgM and IgG antibodies to SARS-CoV-2 develop between 6–15 days post disease onset. The presence of antibodies was detected in <40% among patients within 1 week from onset, and rapidly increased to 100% (total antibodies), 94.3% (IgM) and 79.8% (IgG) from day-15 after onset.

The longevity of the antibody response is still unknown, but it is known that antibodies to other coronaviruses wane over time (range: 12 – 52 weeks from the onset of symptoms) and homologous re-infections have been shown. SARS-CoV-2 IgM and IgG antibody levels may remain over the course of seven weeks or at least in 80% of the cases until day 49. In comparison, 90% and 50% of SARS-CoV-1 infected patients have been shown to maintain IgG antibodies for two and three years respectively. In addition, it could be important to detect nasal IgA antibodies, as the serum IgA antibodies were not raised, but IgA persisted in the nasal mucosa one year post-infection for seasonal coronavirus 229E.

The presence of antibodies against SARS-CoV-2 does not indicate the protection to COVID-19, a correlation between blood antibody levels and immunity has not been established. Long-term serological studies will be required for this purpose.

## 3. TEST PRINCIPLE:

ELISA-VIDITEST anti-SARS-CoV-2 (NP) IgM is a solid-phase immunoanalytical test. The specific antigen (Nucleocapsid Protein) is fixed to each well of the microtiterstrips. Specific antibodies present in the patient's sample are bound during the first incubation step. After removing unbound material by washing, the presence of the specific antibodies is detected using anti-human IgM conjugate during the second incubation. The unbound peroxidase conjugate is then removed and TMB substrate is added, resulting in the development of a blue colour in positive samples. The enzyme reaction is terminated by addition of the stop solution (colour change to yellow). The intensity of the yellow colour thus developed is proportional to the concentration of antibodies in the sample.

## 4. KIT COMPONENTS:

| | |
|---|---|
| ELISA break-away strips in the handling frame coated with the antigen STRIPS Ag | 1 x 12 pcs |
| 1.3 mL Negative control serum, r.t.u. NC | 1 vial |
| 1.3 mL Positive control serum, r.t.u. PC | 1 vial |
| 1.3 mL Calibrator, r.t.u. [1] CAL | 1 vial |
| 13 mL Anti-human IgM antibodies labelled with horseradish peroxidase (anti-IgM Px conjugate) r.t.u. CONJ | 1 vial |
| 2 mL RF sorbent [2], 25x concentrated RF SORB 25x | 1 vial |
| 55 mL Wash buffer, 10x concentrated WASH 10x | 1 vial |
| 60 mL Dilution buffer, r.t.u. DIL | 1 vial |
| 13 mL Chromogenic substrate TMB, r.t.u. TMB | 1 vial |
| 13 mL Stop solution, r.t.u. STOP | 1 vial |
| Instruction manual | |
| Certificate of quality | |

[1] r.t.u., ready to use
[2] Goat anti-human IgG globulin

**Notice:** Control sera may be colorless to yellowish or blue due to the use of different diluents.

**Chromogenic substrate TMB is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB and not compatible with other Chromogenic substrates used in other ELISA-VIDITEST TMB-O, TMB-BF.**

## 5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

Distilled or deionised water for dilution of the Wash buffer concentrate, appropriate equipment for pipetting, liquid dispensing and washing, spectrophotometer/colorimeter (microplate reader – filter with wavelength 450 nm and 620-690 nm reference filter (not required), microtiter plate for pre-incubation of sera with RF sorbent in the VIDIMAT analyzer.

The test can be performed automatically using the VIDIMAT analyzer.

## 6. PREPARATION OF REAGENTS AND SAMPLES:

a. **Allow all kit components to reach laboratory temperature.**
b. **Vortex Solutions, Controls and Peroxidase conjugate in order to ensure homogeneity.**
c. **Vortex sera and plasma samples and Controls well prior use. In the automatic analyzer VIDIMAT the dilution of samples is performed automatically (pre-set dilution for serum and plasma samples is 101x). Prepare Dilution buffer Plus DIL PLUS:** Dilute RF sorbent RF SORB 25x **25x** by Dilution buffer DIL (**i.e. 1 mL RF sorbent + 24 mL Dilution buffer)**. Prepare only an amount necessary for the run, do not store. **When using the VIDIMAT analyzer, consider that you need 300 µl of DIL PLUS per test sample plus 2 ml of reserve. In the case of manual performation** of the test **dilute serum samples 101x in Dilution buffer Plus (e.g. 5 µL of serum sample + 500 µL of Dilution buffer Plus). Incubate 10 min. at laboratory temperature. Dilution buffer Plus contains anti-human IgG antibodies for elimination of IgG antibodies and rheumatoid factor (RF).** Diluted samples may form an opalescent solution. A precipitate does not interfere with the test performance. **Do not dilute** the Controls, they are ready to use.

d. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37 °C in a water bath. Diluted Wash buffer is stable for one month if stored at laboratory temperature.
e. Do not dilute the Controls, Peroxidase conjugate, Chromogenic substrate TMB and Stop solution, they are ready to use.

## 7. ASSAY PROCEDURE:

**Manufacturer will not be held responsible for results if manual is not followed exactly.**

### 7.1 Assay procedure for manual performance

a. Allow the vacuum-closed strips in bag with desiccant to reach a laboratory temperature before opening, to avoid dew condensation. Withdraw the adequate number of strips for reaction. Put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
b. Pipette 100 µL of control sera and diluted tested samples to the wells according to the pipetting scheme (see in Tab 1): start with filling the first well dilution buffer (DIL PLUS), the next two wells with Calibrator CAL, next well with Positive control serum PC and another one well with Negative control serum NC and the remaining wells with tested samples (S1, S2, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the calibrator CAL in triplet and the samples in doublets.

   Incubate **60 minutes** (±5 min) at laboratory temperature.
c. Aspirate the liquid from wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells. Aspirate the contents of the wells and invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
d. Add 100 µL Peroxidase conjugate anti-IgM Px r.t.u. CONJ into each well. Incubate **30 minutes** (±2 min) at laboratory temperature..
e. Aspirate and wash four times with 250 µL/well of Wash buffer as in section c above.
f. Dispense 100 µL of TMB chromogenic substrate into each well. **Incubate 15 minutes** (±30 seconds) at laboratory temperature. **The time measurement must be started at the beginning of TMB dispensing to the first well of the plate.** Follow this rule to avoid breaking the time interval. Pipette quickly at regular rhythm, or use a suitable dispenser. Cover the strips and keep them in the dark during the incubation with TMB substrate.
g. Stop the reaction by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents. Before measuring ensure that the bottom of the wells is clean and the wells of the ELISA plate is without air bubbles.
h. Read the absorbance at 450 nm with a microplate reader **within 20 minutes**. It is recommended to use a reference reading at 620 - 690 nm.

Tab. 1: Pippetting scheme

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | DIL | S4 | | | | | | | | | | |
| b | CAL | S... | | | | | | | | | | |
| c | CAL | | | | | | | | | | | |
| d | PC | | | | | | | | | | | |
| e | NC | | | | | | | | | | | |
| f | S1 | | | | | | | | | | | |
| g | S2 | | | | | | | | | | | |
| h | S3 | | | | | | | | | | | |

### 7.2 Assay procedure for performance on VIDIMAT analyzer

In the automatic analyzer VIDIMAT the entire test is run automatically. For testing serum/plasma samples, please use the appropriate method stated in the **Quality Control Certificate** for specific kit lot.

**Note:** It is necessary to place the microtiter plate for pre-incubation of sera/plasma with RF sorbent in the VIDIMAT analyzer

**Before using the specific kit lot for the first time, it is necessary to set all parameters stated in the Quality Control Certificate for this kit lot.**

In the case of a parallel assay of anti-SARS-CoV-2 (NP) IgM and anti-SARS-CoV-2 (NP) IgG or anti-SARS-CoV-2 (NP) IgA kits, it is necessary to schedule the anti-SARS-CoV-2 (NP) IgM and then anti-SARS-CoV-2 (NP) IgG or anti-SARS-CoV-2 (NP) IgA methods.

The incubation conditions programmed in the software concerned may differ slightly from the specifications given in the instruction manual for the manually performed ELISA-VIDITEST test. These conditions have been validated by the manufacturer. Validation protocols are available on request.

It is possible to perform ELISA-VIDITEST tests using other automatic open analyzers, but this combination must be verified by the user himself.

## 8. PROCESSING OF RESULTS:

**The VIDIMAT analyzer performs the data evaluation automatically.**

Begin the processing of results with subtraction of the background absorbance (absorbance of the DIL PLUS well) from the absorbances of all other wells (controls and tested sera/plasma).
If the absorbance of controls or tested sera/plasma are negative after background subtraction, consider them as zero value.

### 8.1. Processing of results for the Qualitative interpretation

1. Compute the absorbance mean of the wells with Calibrator CAL. If the CAL was applied in three parallels and the absorbance in one well is different from the mean in more than 20%, then exclude the deviating well from the calculation and compute a new absorbance mean using the other two wells.
2. **Compute the cut-off value** by multiplying the mean absorbance of Calibrator with a correction factor. **The correction factor value for this lot is stated in the Quality control certificate**.

Serum samples with absorbances lower than the 90% of the cut-off value are considered negative and samples with absorbances higher than 110% of the cut-off value are considered positive. Serum samples with absorbance in the range 90% - 110% cut-off are equivocal (grey-zone) and then it is recommended to repeat testing or to test another sample from the patient, usually withdrawn 1-2 weeks later.

### 8.2. Processing of results for the Semiquantitative interpretation

Determine Positivity Index for each sample as follows:

1. Compute the **cut-off value** (see the previous paragraph, 8.1, point 2)
2. Compute the Positivity Index according to the following formula:

$$\text{sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}$$

3. Express the serum reactivity according to INTERPRETATION OF RESULTS (see below).

INTERPRETATION OF RESULTS:

| Positivity index | Interpretation |
|---|---|
| < 0.90 | Negative |
| 0.90 - 1.10 | +/- |
| > 1.10 | Positive* |

*on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.

Example of calculation:

| | |
|---|---|
| CAL absorbances | = 1.063; 0.987; 1.025 |
| CAL mean | = 1.025 |
| Sample absorbance | = 0.800 |
| Correction factor | = 0.32 |
| Cut-off value | = 1.025 x 0.32 = 0.328 |
| Sample Positivity Index | = 0.800 / 0.328 = 2.44 |

*Note: An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.*

## 9. CHARACTERISTICS OF THE TEST:

The kit is intended for the qualitative and semiquantitative detection of anti-SARS-CoV-2 IgM antibodies in human serum and plasma. Suitable specimens are serum (heparinised) and plasma samples obtained by standard laboratory techniques. The samples may be heat-inactivated (56°C, 30min), high temperature inactivation does not affect the results.

### 9.1. Validity of the test

The background of the reaction (the absorbance of the Dilution buffer DIL) is less than 0.150.

The mean absorbance values of standards/ control sera, and the ratio between the absorbance values of PC / CAL are in the ranges stated in the Quality control certificate for this kit lot

**10.2. Precision of the test**

The intraassay variability (within the test) and the interassay variability (between tests) were determined by testing samples with different OD values.

**9.2.1 Intraassay variation**

The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot at least on 8 parallels of the same microtitration plate.

Example: (n = number of parallels of the same microtitration plate)

| n | A | ±σ | CV% |
|---|---|---|---|
| 8 | 1.973 | 0.105 | 5.3 % |
| 8 | 1.030 | 0.076 | 7.3 % |

**9.2.2 Interassay variability**

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

Example: (n = number of an independent examination of the same sample):

| n | A | ±σ | min-max | CV% |
|---|---|---|---|---|
| 6 | 1.503 | 0.143 | 1.347-1.768 | 9.5% |
| 5 | 1.140 | 0.140 | 1.015-1.371 | 12.3% |

**9.2.3 Recovery test**

Measured values of recovery test for every Lot are between 80-120% of expected values.

**9.3. Diagnostic sensitivity and specificity of the test**

Diagnostic sensitivity of the test is 93% and diagnostic specificity is 97%. Evaluation was performed on positive and negative patient samples tested with another commercially available diagnostic test (ELISA).

The samples with the equivocal results were not included in the calculation.

**9.4. Interference**

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides, but examination of such a samples is not recommended.
Microbial contaminated specimen may cause interferences.

**10. SAFETY PRECAUTIONS:**

All ingredients of the kit are intended for laboratory use only.

The human sera used in LISA kit have been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. Nevertheless, treat them as infectious material and objects that came into contact with them, autoclave at 121 ° C for 1 hour, or disinfect for at least 30 minutes with a 3% chloramine solution.

Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice, if necessary.

The waste from washing of the strips disinfect a waste container using a suitable disinfectant solution (eg Incidur, Incidin, chloramine,…) at the concentration recommended by the manufacturer.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

## 11. HANDLING PRECAUTIONS:

a. Manufacturer guarantees performance of the entire ELISA kit.

b. Wash solution, TMB chromogenic substrate r.t.u., Stop solution r.t.u. and Dilution buffer r.t.u. are compatible and interchangeable between different ELISA-VIDITEST kits except those with different instruction in its Instruction Manual.

c. Work aseptically to avoid microbial contamination of sera and reagents.

d. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents.

e. Calibrator and control sera contain preservative ProClin 300 (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one a 2-Methyl-2H-isothiasol-3-one (3:1)).

f. Avoid contact of the TMB with oxidizing agents or metal surfaces.

g. Follow the assay procedure indicated in the Instruction manual. Variations in the test results are usually due to:

* Insufficient mixing and prewarming to room temperature of reagents and samples
* Inaccurate pipetting and inadequate incubation times
* Poor washing technique or spilling the rim of well with sample or Peroxidase conjugate
* Use of identical pipette tip for different solutions
* Ccontamination of pipette used for the administration of samples, controls or chromogen substrate solution TMB, Px conjugate (we recommend using pipette which is only reserved for the conjugate application).

## 12. STORAGE AND EXPIRATION:

**The ELISA kit should be used within three months after opening.**

a. Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Under these conditions, the expiration period of the entire kit is indicated on the central label, the expiration date of the individual components is indicated on their package labels.

b. Store unused strips in the sealable pouch and keep the desiccant inside.

c. Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.

d. Store undiluted sera in aliquots at -18 to -28 °C. Frequent freezing and thawing is not recommended. Undiluted serum samples could be stored at +2 to +10°C up to one week.

e. Do not store diluted samples. Always prepare fresh.

## 13. USED SYMBOLS:

 number of tests

CE

Conformité Européenne – product meets the requirements of European legislation

IVD in vitro diagnostics

±σ standard deviation

CV coefficient of variation

OD optical density

 manufacturer

 xpiration

LOT Lot of kit

storage at +2°C to +10°C

°C Celsius degree

% percentage

n number of tested samples

A valuea of tested sample

 read usage instructions

REF catalog number

## 14. FLOW CHART:

| | |
|---|---|
| **Step 1** | **Prepare reagents and samples** |
| | ↓ |
| **Step 2** | **Dispense 100 µL/well controls and samples** |
| | ↓ |
| | **Incubate 60 minutes at laboratory temperature** |
| | ↓ |
| | **Wash 4 times (250 µL/well), aspirate** |
| | ↓ |
| **Step 3** | **Dispense 100 µL/well of peroxidase conjugate** |
| | ↓ |
| | **Incubate 30 minutes at laboratory temperature** |
| | ↓ |
| | **Wash 4 times (250 µL/well), aspirate** |
| | ↓ |
| **Step 4** | **Dispense 100 µL/well of chromogenic substrate (TMB)** |
| | ↓ |
| | **Incubate 15 minutes in dark at laboratory temperature** |
| | ↓ |

| | |
|---|---|
| **Step 5** | **Dispense 100 µL/well of stop solution** |

↓

| | |
|---|---|
| **Step 6** | **Read the absorbance at 450 / 620-690 nm within 20 minutes** |

**General references:**


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