ELISA-VIDITEST
anti-SARS-CoV-2 (RBD) IgG quanti

REF ODZ-497/5ST Σ 96 tests 2°C 10°C 2°- 10 °C

Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative, Quantitative (5ST)

Type samples: Serum/Plasma isolated from venous or capillary blood

Processing: Manual and/or automatic

VIDIMAT

CE IVD

VIDIA

ELISA-VIDITEST anti-SARS-CoV-2 (RBD) IgG quanti

ODZ-497/5ST

Instruction manual

PRODUCER: VIDIA spol. s r.o., Nad Safinou II 365, 252 50 Vestec, Czech Republic, Tel.: +420 261 090 565, www.vidia.cz

1. TITLE:

ELISA-VIDITEST anti-SARS-CoV-2 (RBD) IgG quanti – ELISA kit for the detection of IgG antibodies against the RBD (receptor binding domain) protein, which is part of the spike protein (S1 part) of the coronavirus SARS-CoV-2, causing the disease COVID-19, in human serum and plasma isolated from venous or capillary blood.

2. INTENDED USE:

The test is intended for professional use for the qualitative, semi-quantitative and quantitative detection of IgG antibodies against the RBD (receptor binding domain) protein of the coronavirus SARS-CoV-2 in human serum and plasma. The RBD protein is part of the surface spike protein (S1 part) by which the virus specifically binds to the ACE 2 (angiotensin-converting enzyme 2) receptor of the host cell during infection. If specific antibodies are present in the body (after vaccination with vaccines from different manufacturers and/or after infection), they may bind to the RBD domains of the virus. This blocks their ability to bind to the ACE 2 receptor and neutralizes the virus. Thus, these are highly potent neutralizing antibodies with protective efficacy to prevent the spread of the virus, and therefore most of the currently developed therapeutic anti-SARS-CoV-2 antibodies target the RBD domain of the S protein.

The duration of the antibody response has not yet been confirmed, but the level of antibodies to other coronaviruses is known to decrease over time (ranging from 12 to 52 weeks after the onset of symptoms) and homologous reinfections have been demonstrated. In patients infected with SARS-CoV-1, 90 % of them were shown to persist IgG antibodies for two years and 50 % for three years.

Due to the ongoing pandemic of the SARS-CoV-2 virus, the ELISA-VIDITEST anti-SARS-CoV-2 (RBD) IgG Quanti kit is significantly used in diagnostics, as it allows monitoring of a specific antibody response after infection and/or vaccination, ie. allows monitoring the level of protective and neutralizing antibodies, which is important especially in high-risk groups of patients.

3. TEST PRINCIPLE:

ELISA-VIDITEST anti-SARS-CoV-2 (RBD) IgG quanti assay is a solid-phase immunoanalytical test. The surface of the wells is coated with a specific antigen (recombinant RBD protein). If specific antibodies are present in the test samples, they will bind to the immobilized antigen. After removing unbound material by washing, the bound antibodies then react in the next step with horseradish peroxidase-labeled anti-human IgG antibodies. The unbound peroxidase conjugate is then removed by washing. The amount of bound labeled antibodies is determined by the color

enzymatic reaction of the TMB substrate (blue staining of positive samples). The reaction is stopped by adding of STOP solution (colour change to yellow). The intensity of the yellow colour of the wells depends on the amount of antibodies in the sample.

4. KIT COMPONENTS:

ELISA	break-away strips coated with a mixture of specific antigens STRIPS Ag	1 x 12 pcs.
1.3 mL	Standard A = negative control serum, ST A/NC, r.t.u.[1),2)]	1 vial
1.3 mL	Standard B, ST B, r.t.u.	1 vial
1.3 mL	Standard C, ST C, r.t.u.	1 vial
1.3 mL	Standard D = calibrator, ST D/CAL, r.t.u.	1 vial
1.3 mL	Standard E = positive control serum, ST E/PC, r.t.u.	1 vial
13 mL	Anti-human IgG antibodies labelled with horseradish peroxidase, (Px-conjugate) CONJ, r.t.u.	1 vial
55 mL	Wash buffer, 10x concentrated WASH 10x	1 vial
60 mL	Dilution buffer DIL, r.t.u.	1 vial
13 mL	Chromogenic substrate (TMB substrate) TMB, r.t.u.	1 vial
13 mL	Stop solution STOP, r.t.u.	1 vial
Instruction manual		
Quality Control Certificate		

[1)] r.t.u. ready to use

[2)] The concentration of standards is mentioned in enclosed Quality control certificate (IU/mL, international units/mL)

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 with other Chromogenic substrates 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, thermostat set at 37 °C for ELISA plate incubation, spectrophotometer/colorimeter (microplate reader – filter with wavelength 450 nm and 620-690 nm reference filter (not required)).

The test can be performed automatically using the VIDIMAT analyzer.

6. PREPARATION OF REAGENTS:

a. **Allow all kit components to reach laboratory temperature. Turn on the thermostat to 37 °C.**
b. **Vortex Solutions, Controls and Peroxidase conjugate in order to ensure homogenity.**
c. **Vortex sera and plasma samples and Controls well prior testing. In the automatic analyzer VIDIMAT the dilution of samples is performed automatically (pre-set dilution for serum and plasma samples is 101x). In the case you perform the test manually** dilute the samples **101x** in Dilution buffer and mix (**e.g. 5 µL of serum sample + 500 µL of Dilution buffer**). **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. Withdraw the adequate number of strips for reaction and place them in a frame. Allow the vacuum-closed strips in bag with desiccant to reach a laboratory temperature before opening, to avoid dew condensation. Put unused strips into the provided pouch and seal it carefully with the desiccant kept inside or sealed under vakuum.

b. Choose the proper method for data interpretation (see section 8) and pipette samples according to this. Fill wells with 100 µL of each standard and diluted test sample as follows: Start with filling the first well with 100 µL of Dilution buffer DIL to estimate the reaction background. In case of choosing the qualitative or semiquantitative method, fill two wells with 100 µL/well of Standard D ST D/CAL (serves as calibrator). Next well with Positive control serum ST E/PC and next one well with Negative control serum ST A/NC.

   In case of quantitative method, pipette 100 µL all Standards A-E (ST A/NC, ST B, ST C, ST D/CAL, ST E/PC). Fill the remaining wells with 100 µL of diluted tested samples (S1, S2, S3,…)(see Fig. 1 and/or Fig. 2) as singles, however, if you want to minimize a laboratory error then apply the controls and samples in doublets, in case of qualitative or semiquantitative testing the ST D/CAL in triplet.

   Incubate **30 minutes** (±2 min) **at 37 °C**.

c. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see SAFETY PRECAUTIONS). Wash the wells four times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells. Aspirate the liquid from the wells and invert the plate and tap it on an adsorbent paper.

   Add 100 µL of peroxidase conjugate anti-IgG Px (r.t.u.) into the wells. CONJ. Incubate **30 minutes** (±2 min) **at 37 °C**.

d. Aspirate wells and wash them four times with 250 µL/well of Wash buffer (see point c).

e. Dispense 100 µL of TMB chromogenic substrate into each well. Incubate for **15 minutes** (±30 seconds) **at laboratory temperature**.

   The time measurement must be started after pippeting the first strip 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 with an aluminium foil or keep them in the dark during the incubation.

f. Stop the reaction by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB substrate 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 the plate, make sure that the bottom of the wells is not dirty and that there are no air bubbles in the wells.

g. Read the absorbance at 450 nm with a microplate reader **within 20 minute**s after stopping of reaction. It is recommended to use reference reading at 620-690 nm.

Fig 1: Pippetting scheme (qualitative and semiquantitative evaluation):

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL	S4										
b	ST D/CAL	S…										
c	ST D/CAL											
d	ST E/PC											
e	ST A/NC											
f	S1											
g	S2											
h	S3											

Figure 2: Pipetting scheme (quantitative evaluation):

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL	S3										
b	STA/NC	S…										
c	ST B											
d	ST C											
e	STD/CAL											
f	STE/PC											
g	S1											
h	S2											

7.2 Assay procedure for performance on the VIDIMAT analyzer

When using the VIDIMAT analyzer, sample dilution and the test are performed automatically. To test serum/plasma samples, use the appropriate method mentioned in the **Quality Control Certificate** of the kit lot.

Before the first use of a new lot of the kit, it is necessary to enter all the parameters listed in the Quality Control Certificate of the kit lot.

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

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

8. PROCESSING OF RESULTS

The VIDIMAT analyzer evaluates the test automatically (serum/plasma dilution 101x).

First, begin the processing with subtraction of the absorbance of the DIL well (background absorbance) from the absorbances of controls and test sera/plasma.

If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

8.1 Processing of results for Qualitative interpretation

1. Compute the mean absorbance of Standard D ST D/CAL. If you applied Standard D into 3 wells and if any of the three Standard D absorbances falls out of the range ± 20 % of the mean absorbance then exclude the deviating well from the calculation and compute the mean using the values from the other two wells.
2. **Compute the cut-off value** of the test by multiplication the Standard D mean by the Correction factor. **The Correction factor values for the Standard D determined for this lot of the kit is stated in the Quality control certificate.** Sera that have absorbance value < 90 % cut-off are negative and sera with absorbance value > 110 % cut-off are considered to be positive. Sera with an OD in the range of 90-110 % CO are borderline (gray zone), their testing must be repeated, or another collection from the patient should be examined 1-2 weeks later.

8.2 Semiquantitative evaluation

Determine the Positivity Index for each serum sample as follows:

1. Compute the **cut-off** value as in the previous evaluation (see previous section 8.1 point 2)
2. Compute the Positivity Index for each serum sample by dividing the OD value of the test serum by the cut-off value.
3. Express a serum reactivity according to the Table (INTERPRETATION OF RESULTS)

Table: INTERPRETATION OF RESULTS

Index value	Evaluation
< 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:

Standard D absorbancies	= 1.063; 0.987; 1.025
Mean Standard D absorbance	= 1.025
Sample absorbance	= 0.800
Correction factor of Standard D	= 0.32
Cut-off value	= 1.025*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.

8.3 Quantitative evaluation

Determinations of antibody concentration in the test sample are in international units (IU/mL). International units have been determined according to WHO standard 20/136 and can also be interpreted in units (BAU)/mL (binding antibody units).

Compute the serum/plasma sample antibody concentration in international units (IU/mL) as follows:

1. Construct the calibration curve by plotting the standard units (listed in the enclosed Quality certificate for the kit lot) on the **x**-axis. Plot their average absorbances on the **y**-axis. It is possible to use the logarithmic **x**-axis.

2. Find the place where the absorbance of tested samples intersect calibration curve and find the corresponding values on the axis x. Here, read the antibody titers of the test samples in IU/mL if you dilute the sample 101 times. It is possible to use various softwares for the standard curve fitting and for the calculation of the unknowns, e.g. KimQ, Winliana. For better fitting, the polynomic (four-parameter) function is the most convenient.
3. The calibration curve and standard units are based on a 101x dilution. For other serum dilutions, dilution must be included in the calculation for proper evaluation according to the instructions. Using the calibration curve, we obtain the number of units in the sample (IU / in the sample), which must be converted to IU/mL (according to the following formula):

   (IU / in sample * sample dilution) / (101) = IU/mL

   Example:

   Serum S1 diluted 101x has an OD of 3.600. This OD lies outside the linear part of the curve.

   It is therefore appropriate to dilute the serum 201x - we obtain an OD of eg 1.600; or dilute 401x, we get OD eg 0.800.

   From the calibration curve of the standards we subtract that the value OD = 1.600 corresponds to the value 155 IU, the value OD = 0.800 corresponds to 80 IU. If the sample was diluted 101x according to the previous formula, then:

   155*101/101 = 155 IU/mL

   However, the sample is diluted 201x, so the calculation must be used: 155*201/101 = 308.5 IU/mL

   If the sample is also diluted 401x, the calculation must be used: 80*401/101 = 317.7 IU/mL.

   The IU/mL values obtained from the different sample dilutions by this calculation should correspond to an accuracy of ± 20 %. This applies to the results obtained from the linear region of the calibration curve.

8.3.1. Evaluation of serum/plasma antibody concentration

The evaluation of data in international units for serum/plasma is stated in the Quality Control Certificate of the kit.

Note 1: If the serum being evaluated is within the gray zone, repeat the test. If the serum is again in the gray zone after retesting, repeat the test with an alternative method, or use serum collected from the same individual 1-2 weeks later.
Note 2) Quantification is accurate only in the linear part of the calibration curve. If the measured OD of the sample exceeds the linearity interval (OD 0.200-2.000), it is necessary to repeat the testing of the sample at a higher dilution for accurate quantification.

9. CHARACTERISTICS OF THE TEST:

The ELISA kit is intended for the qualitative, semiquantitative and quantitative detection of anti-SARS-CoV-2 (RBD part of protein S1) IgG antibodies in human serum and plasma. Suitable specimens are serum (heparinised) and plasma samples obtained by standard laboratory techniques. Samples inactivated by high temperatures (56 °C, 30 min) can be used, thermal inactivation does not affect the assay result.

9.1 Validity of the test

The background absorbance (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 ST E/PC / ST D/CAL are in the ranges stated in the Quality control certificate for this kit lot.

9.2 Precision of the test

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

9.2.1 Intraassay variability

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	Mean value	±σ	CV (%)
16	1.504	0.118	8.0 %
10	0.945	0.069	7.0 %

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 serum sample):

n	Mean value	±σ	min – max	CV (%)
9	1.138	0.125	0.995-1.393	11.0 %
9	1.027	0.072	0.919-1.137	7.0 %

9.2.3 Recovery test

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

9.3 Diagnostic specificity and sensitivity

Determination of diagnostic sensitivity and specificity of the test was performed by testing a set of positive and negative samples. From the results, a sensitivity of 99,1 % and a specificity of 100,0 % were calculated. Samples with a borderline result were not included in the calculation.

9.4 Interference

Haemolytic, icteric and lipaemic samples showed no influence on results up to the concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides. Nevertheless, such samples can only be tested with reservations.

9.5 The limit of quantification

The limit of quantification is defined as the lowest measurable concentration which can be 95% sure distinguished from zero. This value is 5 IU/mL.

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-HCV and anti-HIV-1,2. 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.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin, ...) in concentrations recommended by the producer.

Do not smoke, eat or drink during work. Do not pipette by mouth, but by suitable pipetting devices. Wear disposable gloves and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

11. HANDLING PRECAUTIONS

a. The manufacturer guarantees the applicability of the kit as a whole
b. Wash solution, chromogenic substrate TMB r.t.u., Stop solution r.t.u. and Dilution buffer r.t.u. are compatible and interchangeable between different lots of ELISA-VIDITEST, unless otherwise stated.
c. Work aseptically to avoid microbial contamination of serum samples and reagents.
d. Within the collecting, diluting, and storing reagents, proceed to avoid cross-contamination or contamination with enzymatic activity inhibiting agents.
e. Control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3:1)).
f. Avoid contact of the chromogenic substrate 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 of reagents and samples before use
    * inaccurate pipetting and inadequate incubation times referred to in point 7.
    * poor washing technique or spilling the rim of well with sample or Px-conjugate
    * use of identical pipette tip for different solutions or by swapping the caps
    * contamination of pipette which is used for sample, control and chromogen substrate TMB application with Px conjugate (we recommend for conjugate application use of a pipette reserved for this purpose only)

12. STORAGE AND EXPIRATION DATE

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

a. Store the kit and its components in a dry and dark place at +2 to +10 °C. Under these conditions, the expiration date of the entire kit is indicated on the central label on the kit package, expiration of the individual components is indicated on their package.
b. Put unused strips back in the package and seal or seal well with zipper along with desiccant.
c. Kits are transported cooled in thermo bags, transport time up to 72 hours has no effect on expiration. If you notice any serious damage to the packaging of any kit after receiving the kit, please inform the manufacturer immediately.
d. Store unused test sera undiluted in aliquots and frozen at -18 to -28 °C. Frequent freezing and thawing is not recommended. If you store sera at +2 to +10 °C, use them within one week.
e. Solutions of test sera at working concentration cannot be stored. Prepare them always 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

 expiration

LOT Lot of kit

 storage at +2 °C to +10 °C

°C Celsius degree

% percentage

n number of tested samples

A value of tested sample

 read usage instructions

REF catalog number

14. FLOW CHART:

Step 1	**Prepare reagents and samples in working dilution**
	↓
Step 2	**Dispense 100 µL/well of Control and samples**
	↓
	Incubate 30 minutes at 37 °C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3	**Dispense 100 µL/well of Px conjugate r.t.u.**
	↓
	Incubate 30 minutes at 37 °C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4	**Dispense 100 µL/well TMB substrate**
	↓
	Incubate 15 minutes in dark at room temperature
	↓
Step 5	**Dispense 100 µL/well of Stop solution**
	↓
Step 6	**Read optical density at 450 nm/620-690 nm within 20 minutes**

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