



Declaration of Conformity

According to annex III of the Council Directive 98/79/EC on in vitro diagnostic medical device
We,

EKVITESTLAB LLC

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Declare under our sole responsibility that the following in vitro diagnostic medical devices
other than those covered by annex II and devices for performance evaluation

EQUI *Ascaris lumbricoides* IgG - ELISA kit for the qualitative detection of IgG antibodies to *Ascaris lumbricoides*, REF EI-601

Meet the provisions of the Council Directive 98/79/EC concerning medical devices which
apply to them.

Undersigned declares to fulfill the obligations imposed by Annex III section 2 to 5:

- availability of the technical documentation set in Annex III (section 3), allowing the assessment of conformity of the product with the requirements of the Directive.
- the manufacturer shall take necessary measures to ensure that the manufacturing process follows the principles of quality assurance as appropriate for the products manufactured (Annex III section 4).
- the manufacturer shall institute and keep up to date a systematic procedure to review experience gained from devices in the post-production phase and to implement appropriate means to apply any necessary corrective actions (Annex III section 5).

Conformity assessment was performed according to Article 9 (7) and Annex III, section 3.

Our current Quality System is formatted to international standards:

- **ISO 13485:2016 «Medical devices — Quality management systems — Requirements for regulatory purposes»**

Corporate Contact Information

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RESPONSIBLE PERSON'S name: Anna Yurchuk

Position: Director

SIGNATURE :



Date : October 25, 2021

Stamp



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Сертифікат
Certificate

№ Q1M 804 255 C1



Система управління якістю виробника:
Quality management system of manufacturer

Товариство з обмеженою відповідальністю
«ЕКВІТЕСТЛАБ»
«EKVITESTLAB» Limited Liability Company

Місцезнаходження юридичної особи: вул. Велика Васильківська 114, м. Київ, 03150, Україна
Location of the legal entity: 114 Velyka Vasylkivska St., Kyiv, 03150, Ukraine
Фактичне місцезнаходження: Україна, 03057, м. Київ, проспект Берестейський 60/2
Actual location: 60/2 Beresteysky Avenue, Kyiv, 03057, Ukraine

Відповідає вимогам:
meets the requirements of

ДСТУ EN ISO 13485:2018
Вироби медичні. Системи управління якістю.
Вимоги щодо регулювання
(EN ISO 13485:2016, IDT; ISO 13485:2016, IDT
Medical devices – Quality management systems –
Requirements for regulatory purposes)

Сфера застосування:
Scope

Проектування, розробка, виробництво, зберігання та реалізація ІФА-наборів
для діагностики in vitro
Design, development, production, storage and sale of ELISA kits for in vitro diagnostics

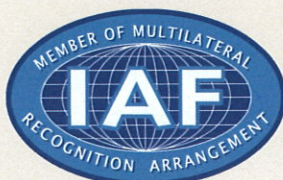
Сертифікат виданий ТОВ «Український Інститут Стандартів», місцезнаходження: будинок 1, вулиця Олександрівська, місто Київ, 03062, Україна.
Атестат акредитації НААУ від 30 червня 2020 року № 80141.
Certificate is issued by LLC Ukrainian Standards Institution: building 1, Oleksandrivska street, Kyiv, 03062, Ukraine.
Accreditation certificate registered on June 30, 2020 No. 80141

Рішення №: 255-000
Decision No.:

Дійсний з: 01.04.2024
Effective date:

Дата видачі: 01.04.2024
Issue date:

Дійсний до: 31.03.2027
Expiry date:



Директор
Director

Наталія СТЕПАНКІВСЬКА
Natalia STEPANKIVSKA

80141
Сертифікація систем
менеджменту

Сертифікат чинний за умови проведення щорічного наглядового аудиту.
Чинність сертифікату необхідно перевірити на офіційному веб-сайті
www.usi.biz.ua або за телефоном: +38-050- 818-7-333
Certificate is valid if the annual surveillance audit has been conducted
The validity of the certificate shall be checked on the official website
www.usi.biz.ua or by tel.: +38-050- 818-7-333

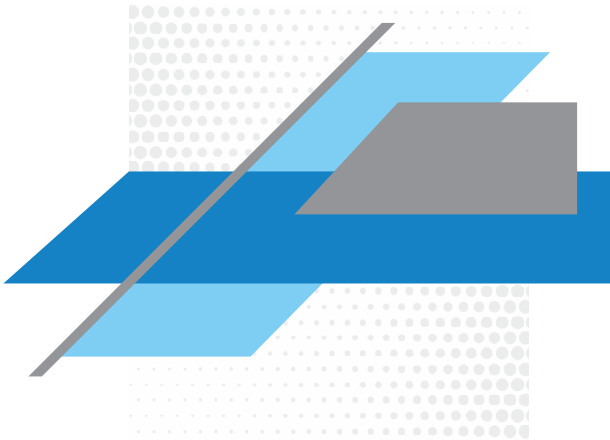


Ascaris

lumbricoides IgG

ELISA kit for the qualitative detection of IgG
antibodies to *Ascaris lumbricoides*

Instructions for use



IVD

REF
EI-601

Σ 96
tests

CE

EQUI *Ascaris lumbricoides* IgG

ELISA kit for the qualitative detection of
IgG antibodies to *Ascaris lumbricoides*

1. INTENDED USE

The «EQUI *Ascaris lumbricoides* IgG» is ELISA kit intended to qualitatively detect anti-*Ascaris lumbricoides* IgG in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose lumbricosis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, rural people, summer visitors.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Ascaris lumbricoides is a human parasite resulting in lumbricosis — one of the most common helminthiases in the world. By some estimates, over a milliard of people infested with acaricides are on earth.

Human ascaris belongs to *Nematoda* roundworms infesting the small intestine of a man who is its exclusive host. *Ascaris lumbricoides* eggs are excreted in the environment with faeces of the infested man. In a warm, wet soil, ascaris larvae develops in the eggs, therefore eggs become invasive only after a maturation period (2 to 3 weeks at 25–30 °C, lower temperatures require longer term). After infestation, larvae leave eggs in the human intestine, penetrates blood circulation and migrate to the liver and lungs with blood flow. The larvae move to the pharynx from the lungs, and here they are re-ingested and further enter the small intestine. In 2 to 3 months, adult ascaris able to propagate develops from larvae in the small intestine.

The helminths are transferred by faecal-oral route upon injection of mature eggs of *Ascaris lumbricoides* with soil-contaminated vegetables, fruits, water, as well as through dirty hands after contact with soil. Lumbricosis is conditionally divided into the early stage (migration of larvae) and late stage (parasitism of adults in the intestine). Invasion is asymptomatic in most cases. Primary feeling of being unwell occurs as early as several days after infestation and is accompanied by weakness, abdominal pain, nausea. Migration of larvae to the lungs may manifest as rales and cough. In some cases, intense invasion may result in pneumonia and liver damage. However, the most common symptom of early lumbricosis are allergic reactions due to hypersensitivity to metabolic products of larvae.

Late stage manifests as decreases appetite, abdominal pain, vomiting, diarrhoea, constipation. Massive ascaris invasion may result in the intestinal obstruction with a lump of helminths or rupture of the walls with peritonitis. When ascarides penetrate other organs, complications may develop such as

hepatitis, cholangitis, pancreatitis and even asphyxia. Cases of neurological disorders sometimes develop in lumbricosis, namely: headache, irritability, sleep impairment, inattention, etc. If no timely treatment is started for intense invasion, it may lead to death, especially in younger children.

Strong immune response to *Ascaris lumbricoides* invasion develops as early as at the early stage. It includes cellular and humoral immunity. Antigens of ascaris larvae stimulate secretion of all-class specific immunoglobulins, however, the level of specific and total IgE antibodies is the highest. The intensity of the immune response (including increased IgG titres) correlates with the massiveness of the invasion.

For diagnosis of lumbricosis, parasitologic stool test for presence of ascaris larvae and eggs is the most common. X-ray imaging of the lungs is additionally applied at the early stage of invasion. Complete blood count (eosinophilia develops in lumbricosis) and detection of serum anti-*Ascaris lumbricoides* antibodies also is included in the set of exams. The presence of specific anti-ascaris antibodies may suggest asymptomatic invasion, and allows initiation of treatment before complications develop in conjunction with other diagnostic instruments.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Ascaris lumbricoides* IgG in «EQUI *Ascaris lumbricoides* IgG» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Ascaris lumbricoides* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Ascaris lumbricoides* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species IgG monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS

1 x 96
wells

Microplate

Each plate well is coated with *Ascaris lumbricoides* antigen. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months

| | | |
|-----------------------|-------------|--|
| CONTROL + | 1 x 0,25 ml | Positive control Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C |
| CONTROL - | 1 x 0,6 ml | Negative control Negative human serum with a preservative (yellow). Store at 2-8 °C |
| DIL SAMPLE | 1 x 13 ml | Serum dilution solution Buffer solution with a milk extract, a detergent and a preservative (brown). Store at 2-8 °C |
| SOLN CONJ | 1 x 13 ml | Conjugate solution (ready to use) Buffer solution of monoclonal antibodies to human IgG, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C |
| SOLN TMB | 1 x 13 ml | TMB solution (ready to use) TMB solution, H ₂ O ₂ , a stabilizer, a preservative (colourless). Store at 2-8 °C |
| TWEEN WASH 20x | 1 x 50 ml | Washing solution TWEEN (20x concentrated) 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days |
| SOLN STOP | 1 x 13 ml | Stop Solution (ready to use) 0.5 mol H ₂ SO ₄ solution (colourless). Store at 2-8 °C |

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620–695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

- do not use the ELISA kit components after the expiry date;
- do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI Ascaris lumbricoides IgG» ELISA kit;
- do not freeze the ELISA kit or its contents;
- after using a reagent, close each vial with its cap;

- when washing, control filling and complete aspiration of solution from the wells;
- use a new pipette tip each time you add samples or reagents;
- prevent direct sunlight from reaching the reagents from the ELISA kit;
- [SOLN|TMB] solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of [SOLN|TMB] with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
- do not use reagents with colour not in line with para. 4.1;
- under no circumstances should the same glassware be used for [SOLN|CONJ] and [SOLN|TMB];
- do not evaluate the test results visually (without a reader);
- any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
- the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

- all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
- conduct the tests in disposable powder-free gloves and goggles only;
- do not eat, drink, smoke, or apply make-up in the test room;
- do not mouth-pipette the solutions;
- controls from the «EQUI *Ascaris lumbricoides* IgG» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
- some of the kit components contain low concentrations of harmful substances and can damage skin or mucosa. In case of contact of [SOLN|TMB], [SOLN|STOP] and [SOLN|CONJ] with mucous membranes or skin, immediately wash the affected area with plenty of water;
- in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

- the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
- the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;

- do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
- disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 °C for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container. Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 °C before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the **STRIPS** for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute **TWEEN|WASH|20x** at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

- 9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.
- 9.2. Fill in the sample application plan.
- 9.3. Prepare the detergent as per para. 8.2.
- 9.4. Add 90 µL of [DIL|SAMPLE] into each plate well.
- 9.5. Add 10 µL of controls and test samples into the wells:
[CONTROL|+] – into well A1,
[CONTROL|-] – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.
- 9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.
- 9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
 - aspirate the content of all wells into a liquid waste container;
 - add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
 - aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
 - repeat the washing step 4 more times;
 - after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.
- 9.8. Add 100 µL of [SOLN|CONJ] into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.
- 9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.
- 9.10. Add 100 µL of [SOLN|TMB] into the wells; do not touch the bottom and the walls of the plate wells.
- 9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.
- 9.12. Add 100 µL of [SOLN|STOP] into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding [SOLN|TMB]. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.
- 9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is impossible, in that case, it is needed to leave one well for blank (only [SOLN|TMB] and [SOLN|STOP] must be added

in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control (\bar{Nc}), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\bar{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \bar{Nc} + 0,3$$

$$IP_{\text{sample}} = OD_{\text{sample}}/CO, \text{ where } OD_{\text{sample}} \text{ is the OD sample.}$$

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

$$\boxed{\text{CONTROL}+} \quad OD \geq 1,0$$

$$\boxed{\text{CONTROL}-} \quad OD \leq 0,150$$

$$\boxed{\text{CONTROL}-} \quad \bar{Nc} \times 0,5 \leq Ncn \leq \bar{Nc} \times 2,0 \quad \text{where } Ncn \text{ is the OD for each } Nc \text{ run}$$

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and \bar{Nc} is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$$\begin{array}{ll} IP_{\text{sample}} > 1,1 & \text{POSITIVE} \\ 0,9 \leq IP_{\text{sample}} \leq 1,1 & \text{BORDERLINE*} \\ IP_{\text{sample}} < 0,9 & \text{NEGATIVE} \end{array}$$

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated in 24 replicates on one series of ELISA kits.

| Sample No. | OD _{av} | IP _{av} | CV, % |
|------------|------------------|------------------|-------|
| 547 | 0,504 | 1,43 | 2,9 |
| 671 | 0,753 | 2,13 | 3,6 |
| 413 | 1,165 | 3,30 | 3,1 |

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

| Sample No. | OD _{av} | IP _{av} | CV, % |
|------------|------------------|------------------|-------|
| 547 | 0,534 | 1,55 | 5,0 |
| 671 | 0,750 | 2,17 | 4,6 |
| 413 | 1,159 | 3,36 | 3,6 |

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 µmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

To evaluate clinical sensitivity and specificity of «EQUI *Ascaris lumbricoides* IgG» ELISA kits, 55 serum samples from patients with clinical symptoms typical for lumbricosis and 60 serum samples from patients without clinical manifestations (seronegative in terms of *Ascaris lumbricoides*) were used. Clinical sensitivity of «EQUI *Ascaris lumbricoides* IgG» ELISA kits was 94.55 % and clinical specificity — 93.3 %.

Method characteristics in comparison with equal commercial ELISA kit was studied in target paediatric population (160 samples) and population of donors (346 samples). For paediatric population serum, relative specificity of «EQUI *Ascaris lumbricoides* IgG» ELISA kits was established at the level of 97.92 % and percent agreement was 95.51 %. For donor population serum, relative specificity of was 89.74 %, relative specificity — 96.30 % and percent agreement was 95.47 %.

12. LIMITATIONS OF ASSAY

Positive result in «EQUI *Ascaris lumbricoides* IgG» ELISA kit supports presence of anti-*Ascaris lumbricoides* specific IgG antibodies. Presence of this class antibodies in newborns is not an evidence of *Ascaris lumbricoides* invasion.

Inconclusive results may suggest a history of *Ascaris lumbricoides* invasion.

Negative result of «EQUI *Ascaris lumbricoides* IgG» ELISA kit supports the absence of anti-*Ascaris lumbricoides* IgG specific antibodies in the test sample or concentration of specific antibodies is below the sensitivity limit of the assay.

The results of serological test only are not the basis for final diagnosis. When establishing the diagnosis, the results of complex laboratory and instrumental tests, as well as clinical manifestations should be considered. Cross-reactions with antibodies to antigens of other helminths cannot be fully ruled out.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

| Possible reasons | Solution |
|---|--|
| <i>High background in all wells</i> | |
| Contaminated washer | Clean the washer head and rinse according to the instructions for use |
| Poor quality or contaminated water | Use purified water with specific resistance $\geq 10 \text{ M}\Omega \cdot \text{cm}$ |
| Use of poorly washed glassware | Use chemically clean utensils |
| Use of chlorinated disinfectants | Do not use chlorine disinfectants |
| Use of contaminated tips | Use new tips |
| Increased incubation times or change in the temperature conditions | Adhere to the incubation regime according to the instructions for use |
| <i>High background in a row of wells</i> | |
| Repeat application of TMB solution | TMB solution should be applied once |
| Contamination of the automatic pipette nozzle with conjugate solution | Clean the pipette and dial carefully liquid |
| Contamination of one of the washer's channel | Clean the flush channel, rinse washer |
| <i>Received OD of the positive control is below the border value</i> | |
| One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added | Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents |
| Reduced incubation times at any stage | Incubate according to instructions for use |
| <i>The colour density of the wells fails to meet the obtained optical density value</i> | |
| This may suggest that the optical beam has been displaced | Check the correct operation of the reader |

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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Manufacturer



Authorized Representative in the European Community



In vitro diagnostic medical device



Catalogue number



Date of manufacture



Use by date



Batch code



Temperature limit



Contains sufficient for <n> tests



Caution



Non-Sterile



Consult instructions for use



Keep away from sunlight



Keep dry



Compliance with EU safety requirements

Edition 8, 10.02.2022

For questions and suggestions regarding the ELISA kit contact:

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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature 18-25°C before use

Dispense 90 µl [DIL|SAMPLE] into the wells (brown)

Add to 10 µl of controls and samples into the wells:
A1 – [CONTROL|+], B1, C1, D1 – [CONTROL|-],
other wells – examined samples
(change of colour from brown to blue)

Cover strips with an adhesive film, incubate for **30 min at 37°C**

Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)

Add 100 µl of [SOLN|CONJ] into all wells (green)

Cover strips with an adhesive film, incubate for **30 min at 37°C**

Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)

Add 100 µl of [SOLN|TMB] into all wells

Incubate for **30 min** in the dark at **18-25°C**

Add 100 µl of [SOLN|STOP] into all wells (change of colour from blue to yellow)

Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$$\bar{Nc} = (Nc1 + Nc2 + Nc3)/3;$$

$$CO = \bar{Nc} + 0,3;$$

$$IP_{\text{sample}} = OD_{\text{sample}} / CO$$

\bar{Nc} - the average value of OD 3-x [CONTROL|-]

CO - Cut off

IP_{sample} - sample positivity index

INTERPRETATION OF RESULTS

| | |
|--|------------|
| $IP_{\text{sample}} > 1,1$ | POSITIVE |
| $0,9 \leq IP_{\text{sample}} \leq 1,1$ | BORDERLINE |
| $IP_{\text{sample}} < 0,9$ | NEGATIVE |