- Determine the concentration (IU/ml) of each investigated sample according to the calibration curve using the absorbance value of each patient sample and control. *If the absorbance value of investigated sample in dilution 1:51 is more than the one of positive control IV (200 IU) it is recommended to investigate this specimen in dilution 1:510. During calculation of IgG concentration of such sample in IU/ml using already drawn calibration curve, obtained result should be multiplied in 10 times, i.e.:*

final concentration = IU/ml x 10

- All suitable available computer programs can be used for automated result reading and calculation.

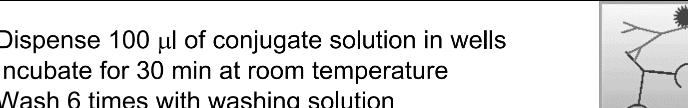
Interpretations

> 20 IU/ml	**positive**
18 – 20 IU/ml	**indetermined**
< 18 IU/ml	**negative**

LIMITATION OF THE PROCEDURE

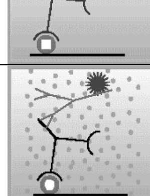
During diagnostics results should be interpreted in conjunction with the patient' s clinical history, symptomatology, the results of others diagnostics procedures as well.

OVERVIEW OF PROCEDURE



- Dispense 100 µl of diluted investigated specimens and controls in wells
- Incubate for 60 min at 37°
- Wash 4 times with washing solution

- Dispense 100 µl of conjugate solution in wells
- Incubate for 30 min at room temperature
- Wash 6 times with washing solution



- Dispense 100 µl TMB substrate in wells
- Incubate for 30 min at room temperature (colouring)
- Stop the reaction by adding 100 µl stop-reagent
- Read the optical density at 450/620 nm

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DIA-Toxo-IgG

Enzyme immunoassay for the quantitative detection of IgG class antibodies against Toxoplasma gondii

96 tests **Product code: T-11G07C**

EXAMPLE FORM

INTENDED USE

Enzyme immunoassay kit is intended for the quantitative detection of IgG antibodies to Toxoplasma gondii in human serum or plasma.

INTRODUCTION

Toxoplasmosis is a protozoan disease caused by Toxoplasma gondii that is obligate intracellular parasite. Contagion with toxoplasmosis may occur at getting products with meat food not enough thermally treated and affected with Toxoplasma cysts. It is possible a transplantation transmission of toxoplasmosis: transplantation of organs from infected donors; and congenital when maternal infection just prior to or during pregnancy via placenta is occurred. In overwhelming majority the acquired toxoplasmosis is taking its latent or chronic form. The latent form of toxoplasmosis is characterized by an asymptomatic course that complicates clinical diagnostics of disease. Today an enzyme immunoassay techniques are widely used for a diagnostic serology of T.gondii for detecting IgG and IgM specific antibodies. At initial infecting IgG antibodies to T. gondii are appeared in blood in 1-2 months and remain in low titers during the term of life. At the recurrence its concentration distinctly rise.

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PRINCIPLE OF PROCEDURE

Main components of the kit are an microelisa strips (immunosorbent) and conjugate. Microelisa strips are coated with Toxoplasma gondii antigen. The conjugate is presented by monoclonal antibodies to human IgG conjugated with the horseradish peroxidase.

When investigated specimens of human plasma or serum are placed into wells IgG antibodies to T. gondii are bound to the antigen on the solid phase. Specific IgG to T.gondii are detected with the conjugate - monoclonal antibodies-HRP.

After washing non-bound components the developer solution (substrate buffer and chromogen TMB) is added into each well.

The peroxidase reaction is stopped by adding the stop-reagent then the absorbance in wells is measured at 450/620 nm, the optical density of mixture is directly proportional to the concentration of IgG antibodies in serum or plasma specimens.
The concentration of antibodies specific to T.gondii can be expressed in International Units (IU) using the calibration curve. Positive controls used for drawing of the calibration curve are standardized in accordance with 3rd WHO International Standard.

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. The kit must not be frozen. Shelf life of the kit is 12 months.

KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

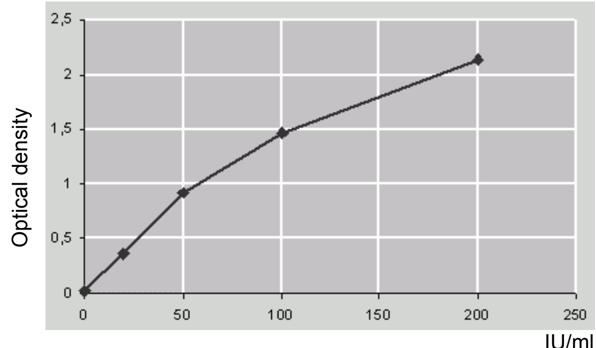
№	Reagents	Presentation
1	**Stock washing solution (№1)** Phosphate buffer, containing 3% Tween 20.	2 bottles 2 × 25 ml
2	**Microelisa strips** 12 strips per plate each with 8 wells coated with T.gondii antigen	1 plate
3	**Solution for sera dilution (№3)** Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.1% sodium azide, 0.01% benzoic acid.	2 bottle 2 × 30 ml
4	**Solution for conjugate dilution (№4)** Phosphate buffer, containing bovine serum albumine. Preservatives: 0.1% MIT, 0.01% benzoic acid.	1 bottle 1 × 15 ml
5	**Substrate buffer** Citrate-phosphate buffer, containing 0.016% hydrogen peroxide.	1 bottle 1 × 8 ml
6	**Chromogen TMB** Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 8 ml
7	**Conjugate** concentrate anti-IgG anti-human IgG monoclonal antibodies conjugated with a horseradish peroxidase. Preservatives: 0.01% thimerosal.	1 vial 1 × 1.5 ml
8	**Negative control (0 IU/ml)** Inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to T.gondii, HIV ½ and HCV. Preservatives: 0.1% sodium azide.	1 vial 1 × 0.2 ml

- Aspirate the contents of all wells and wash the plate with **washing solution** 4 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Add 100 µl of the **conjugate solution** to each well.
- Cover the plate with adhesive film and incubate at room temperature (18-25°C) for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Add to each well 100 µl of the **TMB substrate.**
- Cover the plate with adhesive film and incubate at 18-25°C for 30 minutes in the dark.
- Add to each well 100 µl of the **stop-reagent** (maintain the same pipetting sequence and rate used for TMB substrate addition).
- Read the absorbance at 450/620 nm using a dual wavelength microplate reader within 5 minutes after stopping the reaction.

An absorbance may be measured at 450 nm (single wavelength) against a blank well; in that case include an empty well in the run.

Interpretation of results

- The test performance is considered correct if optical densities of all controls correspond to the following values:
  - The optical density of the **negative control** is not higher than **0.2** OU;
  - The optical density of the **positive control I** is not lower than **0.2** OU;
  - The optical density of the **positive control II** is not lower than **0.5** OU;
  - The optical density of the **positive control III** is not lower than **0.8** OU;
  - The optical density of the **positive control IV** is not lower than **1.2** OU;
- The concentration of specific IgG to T.gondii in each specimen can be expressed in International Units. In order to obtain quantitative results in IU/ml plot the absorbance values of positive controls on a graph paper in a system of coordinates against its corresponding concentrations and draw a calibration curve as shown at the picture below:



Do not use this example (curve) instead of the obtained one in your test performance.

- Reagents and samples should be at room temperature (18-25°C) before testing begins. Return the reagents to 2-8°C after use.
- The temperature in room where performing analysis should be in the range 18-25°C.
- It should accurately dissolve reagents avoiding its contamination.
- Do not perform the test in the presence of reactivity vapours (for example, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- Use glass vessels thoroughly washed and rinsed with deionized water or use disposable ones.
- Do not allow drying contents of wells on all stages of procedure.
- Enzyme reaction is sensitive to metal ions, so avoid contacting with metal elements.
- TMB substrate (solution for chromogen preparation + chromogen TMB) is to be colourless. Appearance of colouring after dilution is evidence of unavailability for using and solution is to be replaced. The solution is to be prepared in clean plastic ware or clean glassware. The reagent is to be kept in dark.
- Prevent the direct light to fall on the working surface during ELISA procedure.
- Use a new tip for brining specimens in wells.
- Never use the same trough for distribution conjugate and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing must be performed strictly according to the instructions, as insufficient plate washing leads to incorrect results.
Use automatic washer*, as recommended; in case of its absence or faulty work – use multi-channel pipette for washing.
Follow this procedure in each washing:
- aspirate the wells contents completely into a waste flask;
- then fill the wells completely with washing solution (not less than 350 μl per well) avoiding overflow of buffer from one well to another;
- aspirate completely.

Make sure that no fluid remains on the top and the bottom of the strips and stripholder after the last aspiration (e. g. by blotting with absorbent tissue).
∗ Contact our company for further information on the different types of washers validated by our technical services.

Test procedure

- Fit the stripholder with required number of **strips**.
- Add 100 μl of preliminary diluted specimens (1:50) and controls (1:50) to wells as follows:
  - well A1: 100 μl of the **positive control I**.
  - well B1: 100 μl of the **positive control II**.
  - well C1: 100 μl of the **positive control III**.
  - well D1: 100 μl of the **positive control IV**.
  - well E1: 100 μl of the **negative control**.
  - well G1, H1, etc.: 100 μl of **specimens**.
- Cover the plate with adhesive film and incubate at 37°C for 60 minutes.

№	Reagents	Presentation
9	**Positive control I (20 IU/ml)** Human serum containing antibodies to T.gondii. Inactivated by heating. Preservatives: 0.1 % MIT.	1 vial 1 × 0.2 ml
10	**Positive control II (50 IU/ml)** Human serum containing antibodies to T.gondii. Inactivated by heating. Preservatives: 0.1 % MIT.	1 vial 1 × 0.2 ml
11	**Positive control III (100 IU/ml)** Human serum containing antibodies to T.gondii. Inactivated by heating. Preservatives: 0.1 % MIT.	1 vial 1 × 0.2 ml
12	**Positive control IV (200 IU/ml)** Human serum containing antibodies to T.gondii. Inactivated by heating. Preservatives: 0.1 % MIT.	1 vial 1 × 0.2 ml
13	**Stop-reagent** 0.5 M sulphuric acid solution.	1 bottle 1 × 15 ml
14	**Adhesive film**	3 items

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- vial for reagents preparation (glass or plastic);
- graduated cylinder (1000 ml);
- absorbent paper;
- sodium hypochlorite solution or other accepted disinfectant;
- sodium bicarbonate;
- ethanol, 70°;
- automatic single-channel pipettes (e.g. 5-40, 20-200, 200-1000 μl) with disposable tips;
- automatic multi-channel pipettes (50-300 μl) with disposable tips;
- incubator, 37±1°C;
- microwell wash system*;
- microwell reader* (with dual wavelength 450/620);
- biohazard waste containers for potentially contaminated materials.

∗ Contact our company for further information on the equipment validated by our technical services.

SAFETY PRECAUTIONS AND WARNINGS

- Use a new tip for pipetting specimens in wells.
- All reagents included in the kit are intended for "in vitro” diagnostic use.
- Wear disposable gloves when handling reagents and samples and thoroughly wash hands after handling them.

- Do not pipette by mouth.
- Human origin material used in the preparation of the negative and positive controls. The positive control has been inactivated by heating. The positive and negative controls were tested and found nonreactive for hepatitis B surface antigen (HBsAg), antibodies HCV and HIV (HIV-1, HIV-2).
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
- Any equipment directly in contact with specimens and reagents as well as the washing solution be considered as contaminated products and treated as such.
- Avoid spilling samples or solution containing samples.
- Spills must be treated with ethyl alcohol 70°. If the contaminating fluid is an acid, spill must be neutralized with sodium bicarbonate and dried with absorbent paper. The materials used for cleaning must be discarded in a contaminated residue container.
- Samples and reagents of human origin, as well as, contaminated material and products must be discarded after decontamination:
  - Either by immersion solid wastes in sodium hypochlorite at a final concentration of 5%, liquid wastes in sodium hypochlorite at a final concentration of 1% during 30 min.
  - Or by autoclaving at 121°C during 2 hours. The best method of inactivating of HIV, HBV, and HCV is an autoclaving.
  - DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE.
- Do not forget neutralize acid solutions before autoclave.
- Avoid any contacts substrate solution, chromogen and stop-reagent with skin and mucous covers.
- The negative and positive controls contain sodium azide as a preservative. Sodium azide may react with laboratory pluming forming copper or lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a huge quantity of water if solutions containing azide are disposed of the sink after inactivation.

SPECIMEN PREPARATION

Serum or plasma specimens are to be stored at 2-8°C during 72 hours. If necessary these specimens may be frozen (not more than two freezing-thawing procedures are allowed) at temperature below –20°C.
All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.
Specimens with sodium azide, hemolysis, hyperlipidemiae or bacterial contamination may not be used in the ELISA procedure.

ASSAY PROCEDURE

Reagents and specimens should be at room temperature (18-25°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use.

Reagents preparation (for 8 wells)

Microelisa strips

Open the bag and remove the strip plate. Return unused strips in the pack. Reseal the pack and store at 2-8°C.
The strips are stable for 4 weeks at 2-8°C after opening the pack.

Washing solution

Check **Stock washing solution (№1)** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37°C.
Dilute the 4 ml of the **stock washing solution (№1)** with 120 ml of the distilled or de-ionised water, shake intensively to dissolve any crystals that may be present.
Washing solution is stable for 10 days at 2-8°C.

Serum dilution (specimens and controls)

Investigated serum samples, positive and negative controls are preliminarily diluted (1:50) with the **solution for sera dilution.** For example, 10 μl of serum is added to 500 μl of the **solution for sera dilution.** Dilution of specimens should be performed before use. *Carefully repipette the mixture.*
Note: Dilution of serum and controls is not to be straight performed in wells of the strip plate. It can be run in separate vessels.

Conjugate solution

Dilute 100 μl of the (10x) **conjugate** with 1 ml of the **solution for conjugate dilution (№4)** (1:10) in a clean vial. Mix well avoiding foaming.
Conjugate solution has to be prepared before use.
Conjugate solution is stable for 6 hours at 2-8°C.

TMB substrate

In order to prepare **TMB substrate**, dilute 0.5 ml of the **chromogen TMB** with 0.5 ml of the **substrate buffer** in a clean vial (1:1). Mix well. TMB substrate must be colourless before use.
The TMB substrate must be kept away from light and no solutions contact with metals or metal ions is allowed.
The solution has to be prepared before use.
TMB substrate is stable for 2 weeks at room temperature (18-25°C) if kept in the dark.

Procedural notes

Authenticity of results depends on correct execution following instructions:
- Reagents should not be used beyond the expiry date shown on the package label.
- Reagents should not be mixed from different lots during performing test.