Version 23-04/24

User's Manual

Toxoplasma gondii IgA Antibody ELISA Kit

REF **DEIA1839**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Toxoplasma gondii IgA Enzyme Immunoassay Kit provides materials for determination of IgA-class antibodies to Toxoplasma gondii in serum.

Principles of Testing

The Toxoplasma gondii IgA ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Microtiter wells as a solid phase are coated with inactivated Toxoplasma gondii antigen. Sample specimens and ready-for-use controls are pipetted into these wells. During incubation Toxoplasma gondiispecific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgA antibodies are dispensed into the wells. During a second incubation this anti-IgA conjugate binds specifically to IgA antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Toxoplasma gondii-specific IgA antibody in the specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with inactivated Toxoplasma gondii antigen.
   (incl. 1 strip holder and 1 cover foil)
2. Sample Diluent ***, 1 vial, 100 mL, ready to use,
   colored yellow; pH 7.2 ± 0.2.
3. IgG-RF-Sorbent***, 1 vial, 6.5 mL, ready to use,
   colored yellow;
   Contains anti-human IgG-class antibody.
4. Pos. Control ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, red cap.
5. Neg. Control ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, yellow cap.
6. Cut-off Control ***, 1 vial, 2.0 mL, ready to use;
   colored yellow, black cap.
7. Enzyme Conjugate **, 1 vial, 20 mL, ready to use;
   colored red,

antibody to human IgA conjugated to horseradish peroxidase.

8. Substrate Solution, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
9. Stop Solution, 1 vial, 14 mL, ready to use;
   contains 0.2 mol/l H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
10. Wash Solution *, 1 vial, 30 mL (20X concentrated for 600 mL), pH 7.2 ± 0.2.
    see "Preparation of Reagents".

* contains 0.03 % ProClin 300

** contains 0.03 % ProClin 300 + 0.01 % Gentamicin sulphate

*** contains 0.03 % ProClin 300 + 0.015 % 5-bromo-5-nitro-1,3-dioxane (BND) + 0.010 % 2-methyl-2H-isothiazol-3-one (MIT)

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450/620nm ±10 nm)(e.g. the CD Instruments Microtiter Plate Reader)
2. Calibrated variable precision micropipettes
3. Incubator 37°C
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Deionised or (freshly) distilled water
7. Timer
8. Absorbent paper

Storage

1. When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
2. Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
3. Opened kits retain activity for four months if stored as described above.

Specimen Collection And Preparation

Serum:

1. Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

Note:Do not use haemolytic, icteric or lipaemic specimens.

2. Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at –20°C prior to assay. Thawed samples should be inverted several times prior to testing.
3. Prior to assaying each specimen is first to be diluted with Sample Diluent. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with IgG-RF-Sorbent.

1) Dilute each specimen **1+25** with Sample Diluent;

   e.g. 20 µL of specimen + 0.5 mL of Sample Diluent. **Mix well.**

2) Dilute this prediluted sample **1+1** with IgG-RF-Sorbent;

   e.g. 60 µL prediluted sample + 60 µL IgG-RF-Sorbent. **Mix well.**

3) Let stand for at least 15 minutes at room temperature, mix well or overnight at 2-8°C and mix well again.
4) Take 100 µL of these pretreated samples for the ELISA.

Note:Controls are ready for use and must not be diluted!

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute Wash Solution **1+19** (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water.

Consumption: ≤ 5 mL per determination.

Crystals in the solution disappear by warming up to 37°C in a water bath.

The diluted Wash Solution is stable for 4 weeks at 2-8°C.

Assay Procedure

Test Procedure

1. Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
2. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
3. Once the test has been started, all steps should be completed without interruption.
4. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
5. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
6. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
7. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
8. After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
9. To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without

splashing accurately to the bottom of wells.

10. During incubation cover microtiter strips with foil to avoid evaporation.

Procedure

Prior to commencing the assay, dilute Wash Solution, prepare samples, mix well before pipette and establish carefully the distribution and identification plan supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder. **Please allocate at least: 1 well (e.g. A1) for the substrate blank, 1 well (e.g. B1) for the Neg. Control, 2 wells (e.g. C1+D1) for the Cut-off Control and 1 well (e.g. E1) for the Pos. Control.** It is left to the user to determine controls and samples in duplicate.
2. Dispense **100 µL** of Neg. Control into well B1, **100 µL** of Cut-off Control into wells C1 and D1, **100 µL** of Pos. Control into well E1 and **100 µL** of each diluted sample with new disposable tips into appropriate wells. Leave well A1 for substrate blank!
3. Cover wells with foil supplied in the kit. Incubate at for 60 minutes at room temperature (20-25°C).
4. Briskly shake out the contents of the wells. Rinse the wells **5 times** with diluted Wash Solution (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense **100 µL** Enzyme Conjugate into each well, **except A1**.
6. Cover wells with foil. Incubate for **30 minutes at room temperature (20-25°C).**

Note: Do not expose to direct sun light!

7. Briskly shake out the contents of the wells. Rinse the wells **5 times** with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add **100 µL** of Substrate Solution into all wells.
9. Cover wells with foil. Incubate for **exactly 10 minutes at room temperature (20-25°C) in the dark.**
10. Stop the enzymatic reaction by adding **100 µL** of Stop Solution to each well. Any blue color developed during the incubation turns into yellow.

Note: Highly positive samples can cause dark precipitates of the chromogen!

11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the Stop Solution.

Measurement

Adjust the ELISA microplate or microstrip reader **to zero** using the substrate blank in well A1. If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results! **Measure the absorbance** of all wells **at 450 nm** and record the absorbance values for each control and sample in the distribution and identification plan. Dual wavelength reading using 620 nm as reference wavelength is recommended. Where applicable **calculate the mean absorbance values** of all duplicates.

Precautions

1. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative

for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

2. Controls and Standards has been found to be non-infectious in cell cultures.
3. Avoid contact with Stop Solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
4. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
5. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
6. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
7. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
8. Do not use reagents beyond expiry date as shown on the kit labels.
9. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
10. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
11. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
12. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.