

Instructions for use

Toxoplasma IgM ELISA Kit

Qualitative assay for anti-*Toxoplasma gondii* IgM antibodies

Product code GD81

96 tests

For *in vitro* research use only

140108

1. Intended use

The Toxoplasma IgM kit is a rapid ELISA designed for the presumptive qualitative determination of IgM antibodies to *Toxoplasma gondii* in human serum or plasma samples. The kit is intended for research use only.

2. Introduction

Toxoplasma gondii is an intracellular protozoan parasite with a worldwide distribution. Although cats are the definitive host, the parasite can infect almost all mammals and birds. Human infection results from ingestion of contaminated soil, careless handling of cat litter, ingestion of raw or undercooked meat or transmission from mother to foetus through the placenta. Serological data indicates that approximately 30% of the population of most industrialised nations are chronically infected with the organism.

Infection with *T.gondii* is asymptomatic in the majority of cases. The most common clinical symptoms of acute toxoplasmosis in the adult are asymptomatic lymphadenopathy, which may be accompanied by fever and malaise, and atypical lymphocytosis symptoms resembling infectious mononucleosis. While serious complications, such as encephalitis, myocarditis and pneumonitis, are rarely seen in the normal host, infection in an immunocompromised host is often fatal.

When a seronegative woman becomes infected with *T. gondii* during pregnancy, the organism is often transmitted to the foetus. Infection during the first trimester may lead to spontaneous abortion, stillbirth, or overt disease in the neonate. Infection acquired later during pregnancy is usually asymptomatic in the neonate, and may not be recognised. Approximately 75% of congenitally infected newborns are asymptomatic. However, nearly all children born with sub-clinical disease will develop chorioretinitis and some may suffer blindness and mental retardation.

Antibodies to the IgM class appear during the first week of primary infection with *T. gondii* and exist only transiently in most patients. Serologic procedures that measure IgM antibodies help identify patients with recently acquired infections.

3. Principle of the test

Test sera are diluted (1:100) with the sample diluent provided. Anti-human IgG is added to the sample diluent sample to eliminate the possibility of interference by antigen-specific IgG and rheumatoid factor, if present. Diluted serum or plasma specimens are incubated for 20 minutes to allow specific antibodies to *T. gondii* to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, *T. gondii* specific IgM is detected using rabbit anti-human IgM conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB/enzyme substrate is added for 10 minutes. A blue colour develops if antibodies to *T. gondii* are present. Addition of stop solution gives a yellow colour and the optical densities of controls, 10 U/ml standard and samples are measured using a microplate reader.

4. Materials included in the Kit

- **Microplate** 96 wells in 12 X 8 break-apart strips, pre-coated with *T. gondii* purified membrane antigen.
- **Reagent 1:** Sample diluent, 46 ml, (blue). Read the instructions before use.
- **IgG absorbent:** Anti-human IgG, 3 x3.5ml. Read the instructions before use.
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- **Reagent 3:** Conjugate (peroxidase conjugated rabbit anti-human IgM), 12 ml, (green). Ready to use
- **Reagent 4:** TMB Substrate, 12 ml. Ready to use
- **Reagent 5:** Stop solution, 12 ml. Ready to use
- **Positive control:** (red), 1ml. Ready to use.
- **10 U/ml standard:** (yellow), 1ml. Ready to use.

- **Negative control:** (green), 1ml. Ready to use.

- **Incubation bag**

- **Instructions for use**

5. Other equipment required

10mm X 60mm tubes for dilution, pipettes 10µl, 100µl, 1000µl; repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device. Distilled or de-ionised water, general laboratory apparatus.

6. Storage and precautions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The 10 U/ml standard and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stop solution contains 0.24M sulphuric acid and is non-corrosive.

7. Samples

Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid haemolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at -20°C. Avoid multiple freeze-thaw cycles.

8. Method

Ensure that all materials are at room temperature before beginning the procedure. We recommend that the 10 U/ml standard and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1. Assemble the number of strips required for the assay.
2. Prepare only sufficient IgG-absorbent-containing sample diluent for the number of samples to be tested. Add one part **IgG absorbent** to 4 parts of **Reagent 1 Sample Diluent** as shown in the examples below and mix thoroughly. Discard any unused IgG-absorbent-containing diluent.

Approx Nr of samples	Volume of sample diluent (ml)	Volume of IgG Absorbent (ml)
24	10	2.5
48	20	5.0
72	30	7.5
96	40	10.0

3. Dilute samples 1:100 (e.g. 5µl serum plus 0.5 ml diluent). It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.
4. Dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells.
5. Place the strips into the incubation bag provided and incubate for **20** minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
6. Dilute the Wash Buffer (**Reagent 2**) 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash

Genesis Diagnostics Ltd, Eden Research Park, Henry Crabb Road, Littleport, Cambridgeshire CB6 1SE, UK.

Tel: +44 (0)1353 862220 Fax: +44 (0)1353 863330 email: genesis@elisa.co.uk web: www.elisa.co.uk

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buffer concentrate to 450ml water. The diluted wash buffer is stable for two months at 2 - 8°C.

- After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing is the key to good results. Blot the wells on absorbent paper before proceeding. **Do not allow the wells to dry out.**

Manual Wash Procedure:

Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process two more times.

- Dispense 100µl of Conjugate (**Reagent 3**) into each well. This reagent is colour coded green. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB substrate reagent! Incubate the wells for **20** minutes in the incubation bag at room temperature.
- After 20 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. **Do not allow the wells to dry out.**
- Using a repeating dispenser, rapidly dispense 100µl of TMB substrate (**Reagent 4**) into each well. Incubate the plate for **10** minutes.
- Add 100µl of Stop Solution (**Reagent 5**) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
- Read the optical density in a microplate reader within 10 minutes.

9. Quality control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

10. Interpretation

Negative samples: OD < 10 U/ml standard OD
Positive samples: OD >= 10 U/ml standard OD

A negative result indicates no current or previous infection with *T. gondii*.

A positive result indicates an active or recent infection with *T. gondii*. The magnitude of the measured result above 10 U/ml standard is not indicative of the total amount of antibody present.

11. Limitations

Results of the Genesis Diagnostics Toxoplasma IgM assay are for research use only

12. Expected values

T. gondii-specific antibodies rise sharply just before or shortly after the onset of symptoms and reach peak titres within one month. *T. gondii*-specific IgM falls to low levels in most patients within 4 to 6 months. In some patients, IgM specific antibodies may be detectable for 8 months to one year.

13. Performance characteristics

Comparative study:

Genesis Diagnostics Ltd, Eden Research Park, Henry Crabb Road, Littleport, Cambridgeshire CB6 1SE, UK.
Tel: +44 (0)1353 862220 Fax: +44 (0)1353 863330 email: genesis@elisa.co.uk web: www.elisa.co.uk
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The Genesis Diagnostics Toxoplasma IgM kit was compared with another commercially available ELISA procedure for the detection of IgM antibodies to *T. gondii*. The results are summarised below.

Comparative Study (n=90)	Reference Toxoplasma IgM ELISA kit	
	+	-
Genesis Diagnostics	6	1
Toxoplasma IgM kit	0	83

14. Assay characteristics

Within Assay Imprecision < 12%
Between Assay Imprecision < 12%

Method Summary

- Mix IgG absorbent and Sample Diluent 1:4 and dilute all samples 1:100
- Dispense 100µl of the 10 U/ml standard, each control and diluted sample into the microplate wells
- Incubate for **20** minutes at room temperature.
- Wash the wells three times*
- Dispense 100µl of Conjugate (**Reagent 3**) into each well
- Incubate at room temperature for **20** minutes
- Wash the wells four times*
- Add 100µl of TMB Substrate (**Reagent 4**) to each well
- Incubate at room temperature for **10** minutes
- Add 100µl Stop Solution (**Reagent 5**) to each well
- Read the optical density at 450nm

Further Reading

Krick JA, and Remington JS: Toxoplasmosis in the adult: An overview. *New Engl J Med* 298: 550-553, 1978
Welch PC *et al*: Serologic diagnosis of acute lymphadenopathic toxoplasmosis. *J Infect Dis* 142:256-264, 1980
Ruskin J, and Remington JS: Toxoplasmosis in the compromised host. *Ann Intern Med* 84: 193-199, 1976
Highes HPA: Toxoplasmosis: The need for improved diagnostic techniques and accurate risk assessment. *Contem Topics Micro Immunol* 120: 10005-139, 1985