

Instructions for use
Toxoplasma IgG ELISA Kit
Qualitative/semi-quantitative assay for anti-*Toxoplasma gondii* IgG antibodies
Product code GD80
96 tests
For in vitro research use only

140108

1. Intended use

The Toxoplasma IgG kit is a rapid ELISA designed for the qualitative/semi-quantitative detection of IgG antibodies to *Toxoplasma gondii* in human serum. The assay is intended to be used to evaluate serologic evidence of previous infection with *T.gondii* and is for *in vitro* research use only. Plasma samples may also be used.

2. Introduction

Toxoplasma gondii is an intracellular protozoan parasite with a world-wide 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 is 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 subclinical disease will develop chorioretinitis and some may suffer blindness and mental retardation.

3. Principle of the test

Diluted serum or plasma specimens (1:100) 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 IgG is detected using rabbit anti-human IgG 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, standard(s) 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** 100mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 10ml, (blue), concentrate (x10)
- **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 IgG), 12 ml, (red), ready to use
- **Reagent 4:** TMB Substrate, 12 ml, ready to use
- **Reagent 5:** Stop solution, 12 ml, ready to use
- **Standards¹:** (for semi-quantitative assays) 50 IU/ml; 150 IU/ml (blue), ready to use
- **Standard¹:** (for qualitative assays) 15 IU/ml, (yellow), ready to use
- **Positive control¹:** 100 IU/ml (red), 1ml, ready to use
- **Negative control:** 1 ml (green), 1ml, ready to use
- **Instructions for use**

¹Calibrated against the 3rd International Standard for Anti-Toxoplasma serum, code TOXM – NIBSC, Potters Bar, UK

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 assay standards 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 standards 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. The sample diluent X10 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 - 8°C. Dilute the Sample Diluent (**Reagent 1**) 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 10ml sample diluent concentrate to 90 ml water.
3. Dilute patient 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. For qualitative determinations, dispense 100 µl of the negative control, the 15 IU/ml standard, the positive control and the diluted patient sample into the wells. For semi-quantitative determinations, use sample diluent as 0 IU/ml and additionally dispense the 50 IU/ml and 150 IU/ml standards.
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 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 red. 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 four 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

Qualitative determinations

Negative samples: OD < 15 IU/ml OD
Positive samples: OD >= 15 IU/ml OD

Semi-quantitative determinations

Plot the optical densities of the standards against their respective concentrations. Draw a line to join the points. Read the concentrations of unknowns from this graph. Concentrations below 15 IU/ml are considered negative; concentrations above 15 IU/ml are considered positive for anti-toxoplasma IgG.

A negative result indicates no current or previous infection with *T. gondii*. Such individuals are presumed to be susceptible to primary infection. However see Limitations below.

A positive result indicates a current or previous infection with *T. gondii*.

11. Limitations

- The antibody titre of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
- Test results for demonstration of seroconversion should be interpreted in conjunction with the clinical evaluation and the results of diagnostic procedures.
- Samples collected too early in the course of the infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion or an IgM specific assay should be performed.
- A positive Toxoplasma IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection.

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
Genesis is a subsidiary of Omega Diagnostics Group plc

12. Performance characteristics

Comparative study:

The Genesis Diagnostics Toxoplasma IgG kit was compared with another commercially available ELISA procedure for the detection of IgG antibodies to *T. gondii*. The Genesis kit showed 100% agreement with the other ELISA. The results are summarised below.

Comparative Study (n=83)	Reference Toxoplasma IgG ELISA kit	
	+	-
Genesis Diagnostics Toxoplasma IgG kit	+	-
	39	0
	0	44

13. Assay characteristics

Within Assay Imprecision < 12%

Between Assay Imprecision < 12%

Method Summary

- Dilute sera 1:100 with sample diluent (**Reagent 1**)
- For qualitative assays, dispense 100µl of the 15 IU/ml standard, the controls and diluted sample into the microplate wells. For semi-quantitative determinations, additionally run the 50 & 150 IU/ml standards.
- 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