11. Assay Procedure Continued…

6. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **20 minutes** at room temperature.

7. After 20 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **10 minutes**.

9. 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.

10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit. Controls are intended to monitor for substantial reagent failure. Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

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.

14. Limitations of the Procedure

1. Results of the Genesis Diagnostics Toxoplasma IgM assay are for research use only
2. *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.

15. Performance Characteristics

Comparative Study
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.

n=90	Reference +	Reference -
Genesis +	6	1
Genesis -	0	83

16. Reproducibility

Within Assay Precision
CV%: <12%

Between Assay Precision
CV%: <12%

17. 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

18. 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

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G·E·N·E·S·I·S Diagnostics

Toxoplasma IgM ELISA Kit

Qualitative assay for anti-*Toxoplasma gondii* IgM antibodies

Product Code: GD081

For Research Use Only. Not for use in diagnostic procedures

Not for sale or use in the EU

1. Materials Included in the Kit

- **Microplate** 96 wells in 12 x 8 break-apart strips, pre-coated with inactivated *T gondii* purified membrane antigen
- **Reagent 1**: **Sample Diluent** 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 46ml, (blue). Read the instructions before use.
- **IgG absorbent:** Anti-human IgG, 3 x 3.5ml. 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** goat anti-human IgM (green) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12ml, ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 10 U/ml (yellow), 1ml of 10mM Tris-buffered saline with human serum IgM antibodies *T. gondii*, ready to use
- **Positive control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to *T. gondii*, 100 U/ml, (red), ready to use.
- **Negative control:** 1ml of 10mM Tris-buffered saline containing normal human serum, (green), ready to use.
- **Instructions for use**

264-081-05

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. 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.

4. Explanation of the Test

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.

5. 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.

6. Safety Precautions

1. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
2. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
3. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
4. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
5. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 - 8°C.

9. Specimen Collection and Storage

Serum and plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

1. 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 Sample Diluent **(Reagent 1)** as shown in the examples below and mix thoroughly. Discard any unused IgG-absorbent-containing diluent.

Approx. # of Samples	Vol. of Sample Diluent (ml)	Vol. of IgG Absorbent (ml)
24	10	2.5
48	20	5.0
72	30	7.5
96	40	10.0

2. 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.

11. Assay Procedure

1. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml IgG-absorbent-containing sample diluent). It is important to dispense all samples, standards and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

2. Assemble the number of strips required for the assay.

3. For qualitative assays, dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells.

4. Incubate for **20 minutes** at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.

5. 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 2 more times.