Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

Toxoplasma IgG Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIATOX1	Toxo IgG Elisa	96 Tests

Intended Use:

The Toxo IgG Elisa is intended to be used for the detection of Toxoplasma IgG antibody in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

During early pregnancy Toxoplasma infection can cause congenital abnormalities in the unborn fetus that may cause premature birth, stillbirth and abnormalities of the central nervous system.
This Elisa assay uses Toxoplasma gondii antigen coated wells. Toxo antibodies present in the sample react with the antigens coated in the wells creating an antigen antibody reaction which is then estimated by the addition of a HRP conjugated anti human IgG and substrate solution. The use of a cut off control and a positive control provides the necessary absorbances to classify patient samples as being positive or negative.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	One 96 well microplate coated with Toxoplasma antigen. Stable when stored at 2-8°C till the expiry date.
Negative Control	1x1ml	Tris Buffer containing salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
Positive Control	1x1ml	Tris Buffer containing Toxoplasma IgG, salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
HRP Conjugate	1x6.5ml	1 vial containing Anti-human IgG conjugated with HRP. Store at 2-8°C. Once opened use within 60 days.
Sample Diluent	1x11ml	Calf Serum for diluting samples. Store at 2-8°C. Once opened use within 60 days.
Wash Solution Concentrate	1x20ml	One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
Substrate A	1x7ml	One bottle containing Hydrogen peroxide in buffer. Store at 2-8°C.
Substrate B	1x7ml	One bottle containing TMB in buffer. Store at 2-8°C.
Stop Solution	1x6ml	One bottle containing H2SO4. Store at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

NOTE 1: Do not use reagents beyond the kit expiration date.

NOTE 2: Avoid extended exposure to heat and light. Opened reagents are stable for 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

NOTE 3: Above reagents are for single 96 well microplate.

Materials required but not provided:

Distilled water, Timer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection, Storage and Stability:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Blood should be collected in plain red top venepuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.
Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, the samples may be stored in temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml of the specimen is required.

Procedure:

Reagent preparation:

1. **Wash Buffer**: Dilute wash buffer concentrate in the ratio of 1:40. (i.e., for every ml of the concentrate add 40 ml of distilled water) Store diluted buffer at 2-30°C for up to 60 days.

TEST PROCEDURE:
Before proceeding with the assay, being all reagents and controls to room temperature (20-27°C)

STEP 1

Preparation: Format the microplate wells for each blank well, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminium bag, seal and store at 2-8°C.

STEP 2

Addition of Sample Diluent: Add Sample Diluent 100ul (2drops) into the appropriate wells except the blank well, positive and the negative control well (blank and positive well set 1 well, negative well set 2 wells).

STEP 3

Addition of Sample: Add 10ul of the sample to the wells mixing it thoroughly with a pipette. Dispense 100ul of Negative and the Positive control to their respective wells. Do not dispense anything into the blank well.

STEP 4

Incubation: Swirl the microplate gently for 20-30 seconds to mix and cover the plate with the plate cover and incubate for 20 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwells. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 6

Addition of HRP Conjugate: Add 1 drop (50ul) of the HRP Conjugate to each well except the blank well. Gently mix the contents of the well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at 37°C.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwells. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 9

Addition of Substrate: Add 1 drop of Substrate A (50ul) and one drop of Substrate B (50ul) into all wells except the blank well. Incubate at 37°C for 10 minutes. Ensure that the incubation is done in the dark.

STEP 10

Stopping the Reaction: Add 1 drop (50ul) of the Stop solution into each well and mix gently. Shake the plate gently for 15-20 seconds to mix, till the solution changes to yellow from blue.

STEP 11

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances. Read the results within 10 minutes of addition of the stop solution. (When using a reader without the facility of a secondary wavelength use the blank well absorbance and subtract this absorbance from the negative, positive and the sample absorbances).

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.

CUT OFF OD: 2.1 x Negative Control OD.

IF THE OD VALUE OF THE NEGATIVE CONTROL IS LOWER THAN 0.09 CALCULATE CUT OFF USING 0.09 ABSORBANCE. IF THE OD VALUE OF THE NEGATIVE CONTROL IS GREATER THAN 0.09 USE THE ACTUAL ABSORBANCE.

Interpretation:

Positive for Toxoplasma IgG: sample OD is >/= Cut off OD
Negative for Toxoplasma IgG: sample OD is < Cut off OD

2. Ratio method:

Positive: Sample OD/Cut off (S/Co) >/= 1
Negative: Sample OD/Cut off (S/Co) < 1

Notes:

1. Do not mix reagents from different batches of reagents.
2. Gently mix the reagents before use.
3. Wash Buffer concentrate may have crystals under different temperature conditions. Ensure that the wash buffer concentrate is homogeneous before use.
4. Keep unused microwells in the original bag.
5. No interference has been found upon addition of 120IU/ml of RF, 80 mg/l of Bilirubin, 3000 mg/dl of Triglycerides or 100 U/ml of EB antibody.
6. Haemolysis with a value greater than 2000 mg/dl of Hb in the sample will cause false positive results. Use samples without haemolysis.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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