Human TOX-IgM(Toxoplasma-Immunoglobulin M) ELISA Kit

V1.1

Catalogue No.: EH4416

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of TOX-IgM in serum, plasma.

Storage: 2-8°C for 6 months.

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
ELISA Microplate(Dismountable)	8×12	2-8°C
Negative Control	1ml	2-8°C
Positive Control	1ml	2-8°C
HRP-conjugated TOX antigen	6ml	2-8°C
TMB substrate A	6ml	2-8°C(Avoid Direct Light)
TMB Substrate B	6ml	2-8°C(Avoid Direct Light)
Stop solution	6ml	2-8°C
Wash Buffer (20 x concentrate)	50ml	2-8°C
Plate Sealer	3pieces	
Instruction manual	1copy	

Principle of the Assay

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This kit was based on Capture enzyme-linked immune-sorbent assay technology. Anti- human IgM (μ chain) antibody was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP-conjugated TOX antigen, if there were any TOX-IgM in the samples, it would form a Anti- human IgM–TOX-IgM–HRP-TOX antigen complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Precautions for Use

1. After opening and before using, keep plate dry.
2. Before using the Kit, balance the reagents at room temperature at least 30 minutes.
3. Storage TMB reagents avoid light.
4. Washing process is very important, not fully wash easily cause a false positive.
5. Don’t let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
6. Don’t reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Washing

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Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (Note: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000×g for 15 minutes. Analyze the serum immediately or aliquot and store at -20°C.
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 minutes at 2-8°C at 1500xg within 30 minutes of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 minutes at 2-8°C at 1000xg. Analyze immediately or aliquot and store frozen at -20°C.
- Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Wash Buffer Preparation:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

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Dilute 50ml Concentrated Wash Buffer to 1000ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

Assay Procedure

Put the kit at room temperature for 20 minutes before use

1. Wash plate 2 times before adding sample and control wells.
2. Label the sample wells, 3 Negative Control, 1 Positive Control and blank well.
3. Add 50 µL of each control or Samples to appropriate wells of the microtiter plate (except the black well).
4. Seal the plate with a cover and incubate at 37°C for 30 min.
5. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1 minute.
6. Add 50ul of HRP conjugated TOX antigen solution into the above wells (controls, test sample). Add the solution at the bottom of each well without touching the side wall.
7. Seal the plate with a cover and incubate at 37°C for 30 min.
8. Remove the cover, and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1 minute.
9. Add 50µl of TMB substrate A and 50µl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 15 min.
10. Add 50µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

Data Analysis

NCx: Mean Absorbance of Negative Control (A)

Cutoff Value = **NCx**+0.10

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Quality control

NCx: A ≤0.08 (If A ≤0.05, set NCx as 0.05. If 0.05＜A ≤0.08, set NCx to the actual value. If A>0.08, it should be retested)

Positive control: A ≥ 0.3

Calculation of Results

1. Sample with absorbance values < Cutoff Value are considered negative.

   Sample with absorbance value ≥ Cutoff Value are considered positive.

2. PCx≤0.3, the test is regarded as invalid, should be tested again.

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