

## Human TPAb(Treponema pallidum antibody) ELISA Kit

**Catalogue No.:** EH4102

**Size:** 96T

**Reactivity:** Human

**Application:** This immunoassay kit allows for the qualitative determination of TPAb in human serum or plasma.

**Storage:** 2-8°C for 6 months.

**NOTE: FOR RESEARCH USE ONLY.**

### Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	8 ×12	2-8°C
TPAb Positive Control	1 vial	2-8°C
TPAb Negative Control	1 vial	2-8°C
HRP-labeled recombinant antigen	1 vial	2-8°C(Avoid Direct Light)
TMB substrate A	1 vial	2-8°C(Avoid Direct Light)
TMB substrate B	1 vial	2-8°C(Avoid Direct Light)
Stop solution	1 vial	2-8°C
Wash buffer (20X)	2 vial	
Plate Sealer	3pieces	
Product Description	1 copy	

### Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275

Fax: (0086)027-87800889

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### Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Recombinant *Treponema pallidum* protein was pre-coated onto 96-well plates. And the HRP-labeled recombinant antigen was used as detection antigen. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP-labeled recombinant antigen, if there were any TPAb in the samples, it would form a TP-TPAb-HRP-labeled TP 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. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. 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

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4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

### Manual Washing

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. Repeat this procedure two more times for a total of TWO washes.

### Automated Washing

Aspirate all wells, wash plate TWO times 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 be set for a soaking time of 1 minute.

### 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 min. Analyze the serum immediately or aliquot and store at -20°C
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

**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.

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## Reagent Preparation and Storage

### Wash Buffer:

Dilute 50 mL Concentrated Wash Buffer into 950 mL Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. 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.

### 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 Controls, 2 Positive Controls and 1 blank well.
2. Add 100 µL of each control or Samples to appropriate wells of the microtiter plate.
3. Seal the plate with a cover and incubate at 37 °C for 60 min.
4. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.
5. Add 0.1 ml of HRP-labeled recombinant antigen into the above wells (controls, test samples). Add the solution at the bottom of each well without touching the side wall.
6. 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.
9. Add 50 µl of TMB substrate A and 50 µl 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 30 min.
10. Add 50 µl 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)

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## Data Analysis

### Calculation of the Cutoff Value

$$\text{Cutoff Value} = \text{NCx} * 2.8$$

NCx (Mean Absorbance of Negative Control). PCx (Mean Absorbance of Positive Control)

when  $\text{NCx} < 0.05$ , Calculate as 0.05.

### Calculation of Results

1. Sample with absorbance values  $<$  Cutoff Value are NON-REACTIVE and are considered NEGATIVE for TPAb.

Sample with absorbance value  $\geq$  Cutoff Value are considered POSITIVE for TPAb.

2. If  $\text{NCx} > 0.1$  or  $\text{PCx} \leq 0.5$ , the test is regarded as Invalid, should be tested again.

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