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Human Treponema Pallidum (TP) Antibodies ELISA Kit

Product Number: ELK015001

Shipping and Storage

1. 2-8℃, stored away from light and moisture
2. Validity period: 6 months

Component

Component	96T	48T
Microplate (96-well, pre-coated)	12 holes×8 strips	12 holes×4 strips
Negative control	0.5mL	0.5mL
Positive control	0.5mL	0.5mL
Detect antibody HRP	10mL	5mL
20× washing buffer	25mL	15mL
Sample diluent	6mL	3mL
Substrate A	6mL	3mL
Substrate B	6mL	3mL
Stop solution	6mL	3mL
Plate Sealer	2 sheets	2 sheets

Description

The reagent kit adopts indirect enzyme-linked immunosorbent assay (ELISA). Add the specimen and HRP labeled detection antibody to the pre coated micro well with Treponema pallidum antigen, incubate and thoroughly wash. Using substrate TMB for color development, TMB is converted to blue under the catalysis of peroxidase and to the final yellow under the action of acid. Measure the absorbance (OD value) at 450nm wavelength using an enzyme-linked immunosorbent assay (ELISA) reader and compare it with the CUTOFF value to determine the presence or absence of Treponema pallidum antibody (TP Ab) in the specimen.

Specimen collection and storage

1. Serum: Use test tubes without pyrogen and endotoxin, avoid any cell irritation during the operation, collect blood, centrifuge at 3000 rpm for 10 minutes, and quickly and carefully separate serum and red blood cells.
2. Plasma: anticoagulant with EDTA, citrate or heparin. Centrifuge at 3000 rpm for 30 minutes and collect the supernatant.
3. Cell supernatant: Centrifuge at 3000 rpm for 10 minutes to remove particles and polymers.
4. Tissue homogenate: Crush the tissue by adding an appropriate amount of physiological saline. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant.
5. Storage: If the sample is not tested in a timely manner after collection, please pack it according to a single dose and freeze it at -20℃ to avoid repeated freezing and thawing. Thaw it at room temperature and ensure that the sample is evenly filled and thawed.

Materials required but not supplied

1. ELISA reader (450nm)
2. High precision sampler and nozzle: 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL.
3. 37℃ constant temperature box.

Note

1. The reagent kit should be stored at 2-8℃ and equilibrated at room temperature for 20 minutes before use. The concentrated

washing solution taken out of the refrigerator will have crystals, which is a normal phenomenon. Heating in a water bath will completely dissolve the crystals before use.

2. The Flat noodles not used in the experiment should be immediately put back into the self sealing bag, sealed (low-temperature drying) and stored.
3. The S0 standard with a concentration of 0 can be considered as a negative control or blank; When operating according to the instructions, the sample has already been diluted 5 times, and the actual concentration of the sample is obtained by multiplying the final result by 5.
4. Strictly follow the time, liquid dosage, and sequence indicated in the instructions for incubation operation.
5. Shake all liquid components thoroughly before use.

Reagent Preparation

20×dilution of washing buffer: Dilute distilled water at a ratio of 1:20, which means adding 19 parts of distilled water to 1 part of 20×washing buffer.

Washing method

1. Manual board washing: Shake off the liquid in the holes, fill each hole with washing solution, let it stand for 1 minute, shake off the liquid in the holes, pat dry on absorbent paper, and wash the board 5 times in this way.
2. Automatic washing machine: Inject 350μL of washing solution into each well, soak for 1 minute, and wash the plate 5 times.

Protocol

1. Take out the required Flat noodles from the aluminum foil bag after 20 min of room temperature balance, and seal the remaining Flat noodles with a self sealing bag and put it back at 4℃.
2. Set up standard wells and sample wells, and add 50μL of standard samples of different concentrations to each standard well;
3. Add 10μL of the test sample to the sample well first, and then add 40μL of sample diluent; Blank holes are not added.
4. Except for blank wells, 100μL of horseradish peroxidase (HRP) labeled detection antibody was added to each well of the standard and sample wells. The reaction wells were sealed with a plate membrane and incubated at 37℃ in a water bath or constant temperature incubator for 60 minutes.
5. Discard the liquid, pat dry on absorbent paper, fill each well with detergent, let it stand for 1 minute, shake off 5% detergent, pat dry on absorbent paper, repeat washing the board 5 times (or use a board washing machine).
6. Add 50μL of substrate A and B to each well, and incubate at 37℃ in the dark for 15 minutes.
7. Add 50μL of termination solution to each well and measure the OD value of each well at a wavelength of 450nm within 15 minutes.

Result judgment

Experimental effectiveness: The average OD value of the positive control wells is ≥ 1.00;

The average OD value of negative control wells is ≤ 0.15.

Cut off calculation: Cut off=Mean value of negative control wells+0.15

Negative judgment: If the OD value of the sample is less than the critical value (Cut off), the sample is negative

Positive judgment: If the OD value of the sample is greater than the critical value (Cut off), the sample is positive.

Test kit performance

1. Accuracy: The average OD value of positive control wells is ≥ 1.00; The average OD value of the negative control wells is ≤ 0.15, indicating the validity of the test results.
2. Specificity: Does not cross react with other soluble structural analogues.
3. Repeatability: The coefficient of variation within and between boards is less than 15%.

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Disclaimers

1. The reagent kit is for research purposes only and should not be used for clinical experiments or mouse experiments. Any consequences arising therefrom shall be borne by the experimenter, and our company shall not be held responsible.
2. Strictly follow the instructions for operation. If the experimenter violates the instructions, the consequences shall be borne by the experimenter.