Human Treponema pallidum (Syphilis) IgG antibody ELISA Kit

Enzyme Immunoassay for the qualitative screening of human IgG antibodies to Treponema pallidum (Syphilis) in serum and plasma.

Catalog number: ARG80614

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. Treponema pallidum antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti-human antibody is added to each well and incubate. After washing away any unbound protein A-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C..
Control A (Negative Control)	2 ml (Ready-to-use)	4°C
Control B (Cut-off control)	2 ml (Ready-to-use)	4°C
Control C (Positive Control)	2 ml (Ready-to-use)	4°C
HRP-antibody conjugate	20 ml (Ready-to-use)	4°C
20X Wash buffer	30 ml	4°C
Sample Diluent	100 ml (Ready-to-use)	4°C
TMB substrate	14 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450/620 nm.
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- After the first opening the kit should be used within 2 months, the diluted wash buffer can be kept for 4 weeks at 4°C.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- If crystals are observed in the 10X Wash buffer, warm up to 37°C until the crystals are completely dissolved.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent

contamination may occur.

- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- Once the test has been started, all steps should be completed without interruption.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

- During incubation cover microtiter strips with foil to avoid evaporation.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately.

Plasma- Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately.

Serum/plasma samples can be stored at 2- 8 °C for up to 5 days. Or aliquot and store samples at ≤-20 °C for longer term storage. Avoid repeated freeze-thaw cycles. Frozen samples only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing. Do not use haemolytic, icteric or lipaemic specimens.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g.: 10 ml of 20X Wash buffer + 190 ml of distilled water). The prepared wash buffer should be stored in 2 – 8 °C and used within 1 month after dilution.

- **Patient sample**: Dilute patient sample **1:101** with **Sample diluent buffer** before assay, mix well. (e.g. 5 µl of serum + 500 µl of sample diluent buffer) Let the diluted samples stand for at least 15 minutes and mix well again prior to assay.

  Note: *the controls are ready-to-use and need not further dilution.*

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20°C to 25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **diluted samples and controls** (1 well for substrate blank; 1 well for negative control; 2 wells for cut-off control; 1 well for positive control) into respective wells.
3. **Cover wells** with foil and incubate for **60 minutes at 37°C**.
4. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **HRP-antibody conjugated** solution into each well **(except substrate blank)**. Cover the plate and incubate for **30 minutes at RT**.
6. **Wash** as according to step 4.
7. Add **100 µl** of **TMB Reagent** into all wells, gently tap the plate to mix well.

Incubate for **15 minutes at room temperature** in the dark.

8. Add **100 µl** of **Stop Solution** to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow. (Note: Highly positive patient samples might cause dark precipitates of the chromogen!
9. Read the OD with a microplate reader at **450/620 nm within 30 minutes** after adding the Stop Solution.

CALCULATION OF RESULTS

1. Adjust the ELISA microplate or microstrip reader to zero using the substrate blank well.
2. If- due to technical reasons- the ELISA reader cannot be adjusted to zero using the substrate blank well, subtract the absorbance value of substrate blank well from all other absorbance values measured in order to obtain reliable results!
3. Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.
4. Dual wavelength reading using 620 nm as reference wavelength is recommended.
5. Where applicable calculate the mean absorbance values of all duplicates.

RESULTS

Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

- Substrate blank: Absorbance value lower than 0.100
- Neg. Control: Absorbance value lower than 0.200
- Cut-off Control: Absorbance value between 0.350 – 0.850
- Pos. Control: Absorbance value between 0.650 – 3.000

Calculation

CO = Mean absorbance value of Cut-off Control

Interpretation

POSITIVE: Patient (mean) absorbance values more than 10 % above CO
(Mean OD patient > 1.1 x CO)

GREY ZONE: Patient (mean) absorbance values from 10 % above to 10 % below CO repeat test 2- 4 weeks later- with new patient samples
(0.9 x CO ≤ Mean OD patient ≤ 1.1 x CO)
Results in the second test again in the grey zone ⇒ NEGATIVE

NEGATIVE: Patient (mean) absorbance values more than 10 % below CO
(Mean OD patient < 0.9 x CO)

Results in Units [U]

$$U = \frac{Sample\ mean\ absorbance\ \times 10}{CO}$$

Cut-off value: 10 U

Grey zone: 9- 11 U

Negative: < 9 U

Positive: > 11 U

ASSAY CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0.69 U/mL- 60 U/mL.

Analytical Sensitivity

The analytical sensitivity of this Treponema pallidum IgG antibody ELISA kit was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 0.69 U/mL (OD 450 = 0.042).

Specificity of Antigen

The antigen used for this Treponema pallidum IgG ELISA shows no cross-reactivity to Fasciola, Strongyloides, Ascaris, Schistosoma, Trichinella and Giarda lamblia. None of the following samples with inferference factors will interfere with the ELISA: samples with HAMA or different ANA, samples with tumor marker (CYFRA), and samples with pregnancy hormones.

Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100%.

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100%.