Human Treponema pallidum (Syphilis) IgM antibody ELISA Kit

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

Catalog number: ARG80615

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. Patient samples are diluted with Sample Diluent and additionally incubated with IgG-RF-Sorbent, containing hyper-immune anti-human IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors. This pretreatment avoids false negative or false positive results. Treponema pallidum antigen has been pre-coated onto a microtiter plate. Controls (ready for use) or pretreated 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 IgM antibody is added to each well and incubate. After washing away any unbound reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IgM antibody 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 IgM antibody 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. Unused strips should be sealed tightly in the air-tight pouch.
IgG-RF-Sorbent	6.5 ml (Ready-to-use)	4°C
Negative Control	2 ml (Ready-to-use)	4°C.
Positive Control	2 ml (Ready-to-use)	4°C.
Cut-off 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 450nm (Optional: read at 610-630 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- 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.
- If crystals are observed in the 20X Wash buffer, warm 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.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- 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.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- During 37°C incubation, it is recommended cover microtiter strips with foil to avoid evaporation.

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. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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 or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. 10ml of 20X Wash buffer + 190 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- Sample: Dilute patient sample 1+50 with sample Diluent. Then dilute the prediluted sample 1+1 with IgG-RF-Sorbent, mix well and incubate 15 minutes at RT.

  Sample Pretreatment:

  1. Dilute each patient specimen 1+50 with Sample Diluent; e.g. 10 µL of specimen + 0.5 mL of Sample Diluent. Mix well.
  2. Thoroughly mix the IgG-RF-Sorbent before use.
  3. Dilute the prediluted sample from step 2 1+1 with IgG-RF-Sorbent (e.g. 60 µL prediluted sample + 60 µL IgG-RF-Sorbent). Mix well.
  4. Let the mixture stand at room temperature for at least 15 minutes and up to a maximum of 2 hours, and mix well again before use.
  5. Take 100 µL of the pretreated samples from step 4 for the ELISA.
- Controls: Controls are ready for use and must not be diluted!

ASSAY PROCEDURE

It is very important that all materials including reagents, samples and controls should be equilibrated to room temperature (RT, 21°C to 26°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 pretreated samples and controls into respective wells. Keep at least 1 well empty as substrate blank. Gently tap the plate to mix well. (It is recommended that samples and controls should be assayed in duplicates, or at least to have 1 well for substrate blank, 1 well for negative control, 2 wells for cut-off control, 1 well for positive control, and 2 wells for each samples)
3. Cover wells with foil supplied in the kit. 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. (Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!)
5. Add 100 µl 1X HRP-antibody conjugated into each well (except substrate blank). Gently tap the plate to mix well. Incubate for 30 minutes at RT in dark.
6. Wash as according to step 4.

7. Add 100 µl of TMB Reagent to each well including substrate blank. Gently tap the plate to mix well. Incubate for exactly 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. Any blue color developed during the incubation turns into yellow.
   Note: Highly positive patient samples can cause dark precipitates of the chromogen!
9. Read the OD with a microplate reader at 450/620 nm immediately. It is recommended read the OD within 30 minutes after adding the Stop Solution.

MEASUREMENT

1. Adjust the ELISA microplate or microstrip reader to zero using the substrate blank.
2. If- due to technical reasons- the ELISA reader cannot be adjusted to zero using the substrate blank, 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.

INTERPRETATION OF 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 - 0.800

Pos. Control: Absorbance value between 0.650- 3.000

- 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 (0.9 x CO ≤ Mean $OD_{patient}$ ≤ 1.1 x CO): repeat test 2- 4 weeks later with new patient samples

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]

Patient (mean) absorbance value x 10/CO = [Units]

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

Interpretation of Results

Cut-off value: 10 U

Grey zone: 9 - 11 U

Negative: < 9 U

Positive: > 11 U