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User's Manual

Treponema Pallidum IgG/IgM ELISA Kit

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Treponema pallidum ELISA Kit provides materials for measurement of antibodies to Treponema pallidum in serum or plasma.

General Description

Treponema pallidum is a species of spirochaete bacterium with subspecies that cause treponemal diseases such as syphilis, bejel, pinta and yaws. The treponemes have a cytoplasmic and outer membrane. They are not seen on a Gram stained smear because the organism is too thin to be observed under a light microscope. The recent sequencing of the genomes of several spirochetes permits a thorough analysis of the similarities and differences within this bacterial phylum. Treponema pallidum subsp. pallidum has one of the smallest bacterial genomes at 1.14 million base pairs (Mb), and has limited metabolic capabilities, reflecting its adaptation through genome reduction to the rich environment of mammalian tissue. The shape of Treponema pallidum is flat and wavy, unlike the other spirochetes, which are helical.

Principles of Testing

The Treponema Pallidum Screen IgG/IgM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Microtiter wells as a solid phase are coated with specific, recombinant treponemal antigens. Sample specimens and ready-for-use controlsare pipetted into these wells. During incubation Treponema pallidum-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated treponemal antigens are dispensed into the wells. During a second incubation this conjugate binds specifically to antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Treponema pallidum-specific antibody in the sample specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells;

Wells coated with Treponema pallidum antigen.

(incl. 1 strip holder and 1 cover foil)

2. High Control *, 1 vial, 2.0 mL, ready to use;

colored yellow, red cap.

3. Low Control *, 1 vial, 2.0 mL, ready to use;

colored yellow, yellow cap.

4. Calibrator*, 1 vial, 2.0 mL, ready to use;

colored yellow, black cap.

5. Enzyme Conjugate *, 1 vial, 13 mL, ready to use,
6. Substrate Solution, 1 vial, 14 mL, ready to use,

Tetramethylbenzidine (TMB).

7. Stop Solution, 1 vial, 14 mL, ready to use,

contains 0.2 mol/L H_2SO_4,

Avoid contact with the stop solution. It may cause skin irritations and burns.

8. Wash Solution *, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 0.1

* contain non-mercury preservative

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450/620nm ±10 nm)
2. Calibrated variable precision micropipettes
3. Incubator 37 °C
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Deionised or (freshly) distilled water
7. Timer
8. Absorbent paper

Storage

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Do not freeze.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been

opened, care should be taken to close it tightly again.

Opened kits retain activity for one month if stored as described above.

Specimen Collection And Preparation

Serum can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note:Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at

room temperature. Do not centrifuge before complete clotting has occurred.

Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time should be frozen only once at –20 °C prior to assay. Thawed samples should be inverted

several times prior to testing.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute Wash Solution 1+19(e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH

value of 7.2 ± 0.2.

Consumption: ~5 mL per determination.

Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved

before use.

The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Assay Procedure

General Remarks

1. Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test

protocol as described.

2. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
3. Once the test has been started, all steps should be completed without interruption.
4. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
5. 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.
6. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
7. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
8. After first opening and subsequent storage check conjugate and control vials for microbial contamination

prior to further use.

9. To avoid cross-contamination and falsely elevated results pipette specimen samples and dispense conjugate without splashing accurately to the bottom of wells.
10. During incubation cover microtiter strips with foil to avoid evaporation.

Assay Procedure

Prior to commencing the assay, the distribution and identification planfor all specimens and controls should be carefully established on a form supplied in the kit.

1. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the substrate blank,

1 well (e.g. B1) for the Low Control,

2 wells (e.g. C1+D1) for the Calibrator and

1 well (e.g. E1) for the High Control.

It is left to the user to determine controls and specimen samples in duplicate.

2. Dispense

100 µLof Low Controlinto well B1

100 µLof Calibrator into wells C1 and D1

100 µLof High Controlinto well E1 and

100 µLof each sample with new disposable tipsinto appropriate wells.

Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for60 minutes at 37 °C.
4. Briskly shake out the contents of the wells.

Rinse the wells 5 times with diluted Wash Solution(300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing

procedure!

5. Dispense 100 µL Enzyme Conjugateinto each well.
6. Cover wells with foil. Incubate for30 minutes at 37 °C.

Do not expose to direct sun light!

7. Briskly shake out the contents of the wells.

Rinse the wells 5 times with diluted Wash Solution(300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

8. Add 100 µLof Substrate Solutioninto all wells.
9. Incubate for exactly 15 minutes at 37 °C in the dark.
10. Stop the enzymatic reaction by adding 100 µLof Stop Solutionto each well.

Any blue color developed during the incubation turns into yellow.

Note: Highly positive specimen samples can cause dark precipitates of the chromogen!

11. Read the optical density at 450/620 nmwith a microtiter plate reader within 15 minutesafter adding the Stop Solution.

Measurement

Adjustthe ELISA microplate or microstrip reader to zerousing the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the

absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbanceof all wells at 450 nmand record the absorbance values for each control and specimen sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance valuesof all duplicates.

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and

incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or CD directly.

Calculation

Mean absorbance value of Calibrator (Calb)

Calculate the mean absorbance value of the two Calibrator measurements (e.g. in C1/D1).

Example:(0.28 + 0.30) : 2 = 0.29 = Calb

Precautions

1. Please use only the valid version of the package insert provided with the kit.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Controls and Standards has been found to be non-infectious in cell cultures.
4. Avoid contact with Stop Solutioncontaining 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
5. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.

6. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
7. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
8. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or

regulation.

9. Do not use reagents beyond expiry date as shown on the kit labels.
10. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
11. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even

of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates

may result slightly different.

12. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.