TREPONEMA PALLIDUM (SYPHILIS) ANTIBODY ELISA KIT

INTENDED USE

This kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of the antibodies to Treponema pallidum (TP) in human serum or plasma. It is intended for screening of blood donors and as an aid for the diagnosis and management of clinical conditions known as syphilis.

SUMMARY

Syphilis is a disease caused by Spirochete bacterium called *Treponema pallidum* (TP). If untreated, the organisms move throughout the body and can cause damage to many organs, making syphilis a life-threatening disease if not treated early enough. People who have been infected with Syphilis experience different symptoms during the 3 stages of the disease. Early, which is defined by the presence of the chancre at the site of inoculation. Syphilis may be further divided into primary, secondary, and early latent syphilis; late syphilis includes late latent and the various forms of tertiary Syphilis. The serological response to syphilis involves production of antibodies to a wide range of antigens, including non-specific antibodies and specific anti-TP antibodies. The first detectable response to infection is the production of specific antitreponemal IgM, which can be detected within 4 to 7 days after the chancre appears and until the end of the second week of infection; antitreponemal IgG appears at about four weeks later. By the time that symptoms develop, most patients have detectable IgG and IgM.

PRINCIPLE OF THE ASSAY

With this kit, the detection of anti-TP antibodies is achieved by antigen "sandwich" enzyme-linked method (ELISA) where polystyrene microwell strips are pre-coated with recombinant Treponema pallidum antigens expressed in E. coli. The sample is incubated in the microwells together with recombinant TP antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. In case of presence of anti-TP in the sample, during incubation the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and the specific antigens-antibody immunocomplex is captured on the solid phase.

After washing to remove sample and unbound conjugates, Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added into the wells. In presence of the antigen-antibody-antigen "sandwich" complex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the sample. Wells containing samples negative for anti-TP remain colorless.

Assay principle scheme: Double antigen sandwich ELISA

Ag(p)+Ab(s)+(Ag)ENZ→ [Ag(p)–Ab(s)–(Ag)ENZ] → blue→yellow (+)
Ag(p) + (Ag)ENZ→ [Ag(p)] → no color (-)

Incubation	Immobilized Complex	Coloring	Results
60min.		15min.	

Ag(p)–pre-coated recombinant Treponema pallidum antigens;
Ab(s)–TP antibodies in sample;
(Ag)ENZ–HRP conjugated recombinant TP antigens;

COMPONENTS

96 Tests

- **MICROWELL PLATE** 1plate

Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. **8 wells×12 strips or 8 strips×12 wells per plate. Each well contains recombinant TP antigens.**

The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return at 2~8℃.

- **NEGATIVE CONTROL** 1vial

Yellowish liquid filled in a vial with green screw cap. 0.5ml per vial.
Protein-stabilized buffer tested non-reactive for TP. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8℃.

- **POSITIVE CONTROL** 1vial

Red-colored color liquid filled in a vial with red screw cap. 0.5ml per vial. Antibodies to TP diluted in protein-stabilized buffer. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8℃

- **HRP-CONJUGATE REAGENT** 1vial

Green-colored liquid filled in a white vial with red screw cap. 12ml per vial. Horseradish peroxidase-conjugated TP antigens. Ready to use as supplied. Once open, stable for one month at 2-8℃.

- **STOCK WASH BUFFER** 1bottle

Colorless liquid filled in a clear bottle with white screw cap. 50ml per b
PH 7.4 20 × PBS. (Containing Tween-20 as a detergent).
DILUTE BEFORE USE -The concentration must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature or for two weeks at 2-8℃.

- **CHROMOGEN SOLUTION A** 1vial

Colorless liquid filled in a white vial with green screw cap. 7ml per vial. Urea peroxide solution. Ready to use as supplied.
Once open, stable for one month at 2-8℃.

- **CHROMOGEN SOLUTION B** 1vial

Colorless liquid filled in a black vial with black screw cap. 7ml per vial. TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8℃.

- **STOP SOLUTION** 1vial

Colorless liquid filled in a white vial with yellow screw cap. 7ml per vial. Diluted sulfuric acid solution (2.0M H_2SO_4).

- **PLASTIC SEALABLE BAG** 1unit

For enclosing the strips not in use.

- **CARDBOARD PLATE COVER** 2sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

- **PACKAGE INSERTS** 1copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.

- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5℃.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

SPECIMEN COLLECTION，TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

2. **Transportation and Storage:** Store samples at 2-8℃. Samples not required for assaying within 3 days should be stored frozen (-20℃ or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8℃, **do not freeze**. To assure maximum performance of this anti-TP kit, during storage protect the reagents from contamination with microorganism o r chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY；FOR PROFESSIONAL USE ONLY！**

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30℃) before use.
4. Shake reagent gently before, and return to 2-8℃ immediately after use.
5. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
6. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
7. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
8. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
9. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
10. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37℃ inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers

should be collected and autoclaved for 1hour at 121℃ or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.

18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

ASSAY PROCEDURE

Step1 **Reagents preparation:** Allow the reagents and samples to reach room temperature **(18-30°C)**for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37℃ until crystals dissolve**.** Dilute the stock Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative controls **(e.g. B1, C1, D1),** two Positive controls **(e.g. E1, F1)** and one Blank (**e.g.A1,** neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step3 **Adding HRP-Conjugate:** Add **100µl** HRP-Conjugate into each well except the Blank.

Step4 **Adding Sample:** Add **20µl** of Positive control, Negative control, and specimen into their respective wells - the HRP-Conjugate-sample mixture in the wells will change the color from **GREEN** to **BLUE** after adding of the samples. **Note: Use a separate disposal pipette tip for each specimen, Negative, Positive Control to avoid cross-contamination.**

Step5 **Incubating:** Mix by taping the plate gently. Cover the plate with the plate cover and incubate for **60minutes at 37°C**.

It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step6 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **6times** with diluted Wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap out any remainders.

Step7 **Coloring:** Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, cover the plate with plate cover and mix gently by tapping the plate. Incubate the plate at **37 ℃ for 15 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-TP positive sample wells.

Step8 **Stopping Reaction:** Remove and discard the plate cover. Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensive yellow color develops in positive control and anti-TP Positive sample wells.

Step9 **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within 5 minutes after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of Cut-off value (C.O.) = *Nc + 0.18

Example:
1. Calculation of Nc:

Well No	B1	C1	D1
Negative controls OD value	0.032	0.031	0.027

Nc=0.030
2. Calculation of Cut-off value (C.O.)= 0.030 +0.180= 0.210

*Nc = the mean absorbance value for three negative controls

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:

The test results are valid if the Quality Control criteria are verified. It is advisable that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1)： Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no anti-TP antibodies have been detected with this kit. Therefore, the patient is probably not infected and there are no serological indications for past infection with TP.

Positive Results (S/C.O. ⩾ 1)： Samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive ,which indicates that TP antibodies have been detected using this anti-TP ELISA kit.

Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples can be considered positive for antibodies to Treponema pallidum and therefore there are serological indications for current or past infection with TP. Any blood unit containing antibodies to Treponema pallidum should be immediately discarded.

Borderline (S/CO =0.9-1.1)： Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results. Repeatedly positive samples could be considered positive for antibodies to TP.

Retesting of these samples in duplicates is recommended. Repeatedly positive samples could be considered positive for Treponema pallidum. Follow-up and supplementary testing any positive with other analytical system is required.

TEST PERFORMANCE AND EXPECTED RESULTS

The clinical performances of this assay have been evaluated by a panel of samples obtained from 3400 healthy blood donors from 3 blood banks and by a panel of samples from 192 Syphilis positive patients (comparative study with another commercially available TP diagnostic systems). The evaluation results are given below. Results obtained in individual laboratories may differ.
Results of healthy Samples:

Samples	**-**	**+**	**Confirmed positive**	**Specificity**	**False positives**
Donors: 3400	3392	8	5	99.91%	3

Results of Know Positive samples:

Samples		**anti-TP ELISA***		**TRUST /RPR**		**TPHA**		**anti-TP ELISA***	
Syphilis	No	+	-	+	-	+	-	+	-
1st period	76	70	6	65	11	69	7	68	8
2nd period	110	110	0	101	9	108	2	109	1
3rd period	2	2	0	2	0	2	0	2	0
latent period	4	4	0	3	1	4	0	4	0
TOTAL	192	186	6	171	21	183	9	183	9
Atrophic arthritis	24	0	24	13	11	0	24	3	21
	Control panel								

Anti-TP ELISA*-another commercially available anti-TP ELISA kit

Analytical Specificity:

No cross reactivity was observed with specimens from patients infected with HAV, HCV, HBV, HTLV, CMV, and HIV. No interference was observed from rheumatoid factors up to 2000U/ml .No high dose hook effect observed during clinical testing. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

Reproducibility		**Within run**		**Between run**	
Specimen Type	N	Mean S/CO	CV	Mean S/CO	CV
Weak positive	10	3.35	8.4%	3.23	9.0%
Moderate positive	10	6.75	7.0%	6.40	7.5%
Strong positive	10	10.90	4.2%	10.30	4.4%

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA method. The test is designed to achieve very high performance characteristics of high sensitivity and specificity.
2. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
3. If, after retesting of the initially reactive samples, the assay results are negative , these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antibodies concentrations.

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

1. Values of the Positive or Negative controls ,which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

VALIDITY

Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels

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