Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

Syphilis (TP) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIASYP1	Syphilis (TP) Elisa	96 Tests

Intended Use:

The Syphilis (TP) elisa assay is intended for the qualitative determination of antibodies to Treponema pallidum 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. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

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.

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.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with recombinant TP antigens. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2-8°C.
Negative Control	1x0.5ml	Protein stabilized buffer tested non-reactive for anti TP-antibodies. Yellow in colour. Ready to use. Once open stable for 1 month at 2-8°C.
Positive Control	1x0.5ml	Protein stabilized buffer dilution of anti TP antibodies. Red coloured solution. Once open, stable for 1 month at 2-8°C.
HRP-Conjugate	1x14ml	Green coloured liquid. HRP conjugated recombinant TP antigens. Once open, stable for one month at 2-8°C.
Wash Buffer (20X)	1x50ml	PBS at pH 7.4. 20X concentrate. Once open, stable for one month at 2-8°C.
Chromogen A	1x8ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Chromogen B	1x8ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	1x8ml	Diluted Sulfuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials provided but not required:

Distilled water or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing systems, disposable pipette tips, absorbent tissue or clean towel, dry bath incubator or water bath, plate reader, single wavelength 450nm or dual wavelength 450/630nm and microwell aspiration systems.

Specimen Collection:

1. No special patient preparation is required. Collect the specimen in accordance with normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venepuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimens should be removed by centrifugation at 3000 RPM for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric or haemolytic specimens should not be used as they give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. The Prestige Syphilis TP Elisa assay is used only for testing individual serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled (mixed) blood.
4. Transportation and Storage: store specimens at 2-8oC. Specimens not required for assaying within 7 days should be stored at -20oC or lower. Multiple free thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure 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 for optimal performance of the tests.**
2. **Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.**
3. **CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.**
4. **Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.**
5. **Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.**
6. **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.**
7. **Avoid assay steps long time interruptions. Assure same working conditions for all wells.**
8. **Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.**
9. **Assure that the incubation temperature is 37°C inside the incubator.**
10. **When adding specimens, do not touch the well's bottom with the pipette tip.**
11. **When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.**
12. **The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.**
13. **If using fully automated equipment, 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.**
14. **All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.**
15. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, 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. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.**
16. **Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.**
17. **Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.**
18. **The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121oC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request.**
19. **Some reagents may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Contact with skin and the mucosa should be avoided but not limited to the following reagents: stop solution, chromogen reagents and the wash buffer.**
20. **The stop solution contains sulfuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
21. **Proclin 300 is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
22. **INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS: The values of positive and negative controls which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and or operator or equipment errors. In such cases, the results should be considered as invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration or instability of the reagents, immediately discard the reagents in use and use a new kit. Contact the local Prestige representative.**

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (18-30°C). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize by warming at 37°C, until crystals dissolve. Dilute the wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and clean vessels to dilute the buffer. All other reagents are ready to use as supplied.

STEP 1

Preparation: Mark 3 wells as Negative controls (e.g. B1,C1,D1), 2 wells as Positive controls (e.g. E1,F1) and one Blank (e.g.A1 – taking care that neither the HRP conjugate nor any samples should be added to the blank well). If the results are read using a plate reader having dual wavelength (450 / 630nm) then the Blank well need not be used. Use the required number of strips for the test.

STEP 2

Addition of the sample and HRP conjugate: Add 100ul of HRP-Conjugate into each well except the blank. Add 20ul of Positive control, Negative Control and specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip

for each specimen, negative control and positive control to avoid cross-contamination. Mix by tapping the plate gently.

STEP 3

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 4

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 5

Addition of the chromogens: Add 50ul of Chromogen A and 50ul of Chromogen B into each well including the blank. Incubate the plate at 37oC for 15 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP conjugate produces blue colour in Positive control and Anti TP Positive samples.

STEP 6

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Intensive yellow colour develops in the positive control and Anti TP positive sample wells.

STEP 7

Measurement: 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: Absorbances must be read within 10 minutes of adding the stop solution).

Instructions for Washing:

- To remove any effect washing on false positive reactions, a 5 automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash. This helps in avoiding false positive reactions and a high background.
- To avoid cross-contamination of the plate with specimen or HRP conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
- 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.
- In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400ul/well and aspirating the liquid 5 times. If poor results are observed with high background, increase washing cycles to soak time per well.
- Treat the liquid aspirated after the reaction from the wells with Sodium hypochlorite (at a concentration of 2.5%) for 24 hours before they are disposed off in the appropriate way.
- The concentrated wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

Calculation of results:

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) with 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 A from the absorbances of the specimens and the controls. In case the results are based on a dual filter plate reader, do not subtract the blank value A from the specimen and controls absorbances.

Calculation:

Cut off value (C.O) = Nc +0.18

(Nc = the mean absorbance value for 3 negative controls)

Validation:

Blank well: the absorbance must be <0.080 at 450nm.

Positive Control: the absorbance must be >/= 0.800 at 450/630nm 0r at 450nm after blanking.

Negative control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the other two values. If more than one negative control absorbance does not meet the criteria, the test is invalid and must be re-tested.

Example:

Blank Value	A1: 0.025	450nm (blanking is required only when reading with a single filter)	
Negative control	B1	C1	D1
	0.020	0.012	0.016
Positive Control	E1	F1	
	2.421	2.369	

Calculation of Nc: ((0.016+0.012+0.016)/3) = 0.016

Calculation of the cut off: 0.016 + 0.18 = 0.196

Interpretation of the results:

Negative Results: (A/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: (A/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: (A/C.O = 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.

Follow up, confirmation and supplementary testing of any positive specimen with other analytical systems is required. Clinical diagnosis should not be established using a single result.

- After re-testing of the initially reactive samples, both wells show negative results (A/C.O <0.9). These samples should be considered negative and the original result must be classified as false positive. As with many sensitive Elisa Assays, false positive results can occur due to several reasons, most of which are connected with, but not limited to inadequate washing step.
- After retesting in duplicates one or both wells show positive results. The final result of this specimen should be recorded as repeatedly positive. Repeatedly reactive specimens could be considered positive for anti TP and the blood unit must be discarded.
- After re-testing in duplicates, samples with values close to the cut-off should be interpreted with caution and considered borderline zone sample, or uninterpretable for the time of testing.

Performance Characteristics:

Clinical Specificity: The clinical specificity of this assay was determined by a panel of samples from >4000 healthy donors. The sensitivity of the assay was found to be 99.52% (413 / 415) while the specificity was 99.95% (3859 / 3861).

Serum samples collected from 222 syphilitic patients who had been diagnosed correctly (which included all the periods of the disease), 42 autoimmune diseases patients excluding syphilis (RPR positive) and 270 healthy blood donors were tested for antibody against the Treponema pallidum by RPR, TPHA and TP-Elisa methods. The positive rates of TP Elisa, TPPA and RPR for detection of antibody against treponema pallidum were 97.3% (216/222), 95.95% (213/222) and 90.54% (201/222) respectively. There was no statistical difference between TPPA and TP Elisa for the diagnosis of syphilis. (P>0.05). False positives did not appear in TPPA, TP Elisa methods for the 42 autoimmune patients.

Analytical Specificity: No cross reactivity with HAV,HCV, HIV, CMV and HBV

No interference from RF up to 2000 IU/ml.

No high dose hook effect observed during clinical testing.

The assay performance characteristics are unaffected from elevated concentration of bilirubin, haemoglobin and triolein.

More details on performance characteristics can be requested from our technical department.

Limitations:

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Antibodies may be undetectable during the early stages of the disease and in immunosuppressed individuals. Negative results are only an indication that the sample does not contain detectable levels of anti-Teponema antibodies and should not be considered as a conclusive evidence that the individual is not infected with TP.
- If, after re-testing of the initially reactive specimens, the assay results are negative, these samples should be considered as non-repeatable and interpreted as negative. As with many sensitive Elisa assays, false positive reactions occur due to several reasons most of which are related to but not limited to inadequate washing step.
- The most common assay mistakes are: using kits beyond expiry dates, bad washing procedures, contaminated reagents, improper operation with equipment, sample collection issues.
- The prevalence of the marker will affect the assay's predictive values.
- This assay cannot be utilized to test pooled plasma. This kit can only be used with individual serum or plasma samples.
- This assay is a qualitative assay and the results cannot be used to measure antibody concentrations.

References:

1. Fraser CM, et al. Complete genome sequence of Treponema pallidum, the syphilis spirochete. Science 1998; 281:375.
2. Holmes KK, Lemon SM, Mardh P, Piot P, Sparling PF, Stamm WE, Wasserheit JM, Weisner PF. Chapters 33-36. In Sexually transmitted diseases, 3rd ed. New York: McGraw-Hill,1999.
3. Hook EW III, Martin DH, Stephens J, Smith BS, Smith K. A randomized, comparative pilot study of azithromycin versus benzathine penicillin G for treatment of early syphilis. Sex Transm Dis 2002 Aug; 29(8):486-490.
4. Hook EW III, Stephens J, Ennis DM. Azithromycin compared with penicillin G benzathine for treatment of incubating syphilis. Ann Intern Med 1999 Sept 21; 131(6):434-437.
5. Johns DR, Tierney M, Felsenstein D. Alteration in the natural history of neurosyphilis by concurrent infection with the human immunodeficiency virus. N Engl J Med 1987; 316:1569-72

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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