

# Syphilis ELISA Kit

For the detection of IgG/IgM type antibodies to treponema pallidum in human serum or plasma.

Catalogue Number: EL10017

*96 tests*

FOR LABORATORY RESEARCH USE ONLY.  
NOT FOR DIAGNOSTIC USE.



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## INTENDED USE

This Syphilis ELISA kit is to be used for the *in vitro* detection of IgG/IgM type antibodies to *Treponema pallidum* in human serum or plasma. This kit is intended for LABORATORY RESEARCH USE only, and is not for use in diagnostic or therapeutic procedures.

## INTRODUCTION

Syphilis is a complex sexually transmitted disease that is caused by the spirochaete *T. pallidum*. The disease evolves through primary, secondary and latent stages. Tertiary manifestations of syphilis may occur from one to thirty years after primary infection and include the development of gummatous lesions, cardiovascular syphilis and neurological syphilis. *T. pallidum* cannot be cultured *in vitro* and therefore diagnosis is dependent on clinical signs, direct observation of the bacteria from lesions, and serology. Except for very early disease, serological procedures are the preferred diagnostic method for syphilis at all stages. Two types of serological tests are used: non-treponemal and treponemal. Both non-treponemal and treponemal serology as well as clinical history is required for definitive diagnosis.

Non-treponemal tests detect antibodies reactive with a lipoidal antigen consisting of cardiolipin, lecithin and cholesterol. These non-treponemal antibodies are thought to be generated in response to lipid associated with the treponeme cell surface. The non-treponemal tests may be used for screening and to monitor the effectiveness of therapy. Treponemal tests detect antibodies reactive with whole *T. pallidum* or sonicates thereof. Typically, sera must be adsorbed with an extract of non-pathogenic treponemes to remove cross-reactive antibodies prior to performing treponemal tests. Treponemal tests become reactive after primary infection and in most cases remain positive for the lifetime of the patient. Test sensitivity varies with the stage of syphilis (primary 69-100%, secondary 100%, latent 97-100%, and late 94-96%). False negative results are associated with early primary disease and prozone reactions can occur in the agglutination-based treponemal tests. Treponemal tests have a specificity of 94-100% in normal populations.

The *T. pallidum* antigen has been identified as an abundant, immunodominant and putatively pathogen-specific membrane lipoprotein of *T. pallidum*. Antibodies to the *T. pallidum* antigen protein have been identified in patients with syphilis and in infants with congenital syphilis by immunoblot and by enzyme immunoassay.

## PRINCIPLE OF THE ASSAY

This Syphilis enzyme linked immunosorbent assay (ELISA) kit employs a technique called a qualitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with highly purified specific *T. pallidum* antigen. Samples or controls are added to the microtiter plate wells and incubated. *T. pallidum* IgG/IgM specific antibodies if present, will bind to and become immobilized by the antigen pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound other components of the sample. A standardized preparation of horseradish peroxidase (HRP) conjugated goat anti-human IgG/IgM antibodies is added to each well to “sandwich” the antibodies immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP conjugate and a TMB (3,3',5,5' Tetramethyl-benzidene) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain *T. pallidum* IgG/IgM specific antibodies and HRP conjugate will exhibit a change in colour. This enzyme substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. Samples with O.D values greater than or equal to the Cut-off Value are considered reactive by the criteria of this Syphilis ELISA Kit.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

<b>96 tests</b>
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1. **MICROTITER PLATE** (Part EL17-1) \_\_\_\_\_ **96 wells**  
Pre-coated with *T. pallidum* antigen.
2. **CONJUGATE** (Part EL17-2) \_\_\_\_\_ **0.2 mL**  
Horseradish peroxidase conjugated goat anti-human IgG/IgM antibodies (100-fold concentrated).
3. **BLANK CONTROL** (Part EL17-3) \_\_\_\_\_ **1 mL**  
Buffered solution with animal serum and preservative. (GREEN COLOURED).
4. **NON-REACTIVE CONTROL** (Part EL17-4) \_\_\_\_\_ **1 mL**  
Inactivated normal human serum diluted in sample diluent (YELLOW COLOURED).
5. **REACTIVE CONTROL** (Part EL17-5) \_\_\_\_\_ **1 mL**  
Inactivated human *T. pallidum* antibody positive serum diluted in sample diluent (RED COLOURED).
6. **SAMPLE DILUENT** (Part EL17-6) \_\_\_\_\_ **15 mL**  
Buffered solution with animal serum and preservative. (BLUE COLOURED).
7. **CONJUGATE DILUENT** (Part EL17-7) \_\_\_\_\_ **20 mL**  
Buffered solution with animal serum and preservative. (RED COLOURED).
8. **WASH BUFFER (20X)** (Part 30005) \_\_\_\_\_ **60 mL**  
20-fold concentrated solution of buffered surfactant.
9. **SUBSTRATE A** (Part EL17-8) \_\_\_\_\_ **10 mL**  
Buffered solution with H<sub>2</sub>O<sub>2</sub>.
10. **SUBSTRATE B** (Part 30007) \_\_\_\_\_ **10 mL**  
Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30007) \_\_\_\_\_ **7 mL**  
2 N sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). Caution: Caustic Material!

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 5-100 $\mu$ L and 50-200 $\mu$ L for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Incubator (37°C  $\pm$  2°C).
8. Microtiter plate reader (450 nm  $\pm$  2nm)
9. Automatic microtiter plate washer or squirt bottle.
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water.
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.

## PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Controls, conjugate and microtiter plates are matched for optimal performance. Use only reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water bath to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum, plasma and the Controls in the Kit should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All materials should be disposed of in a manner that will inactivate human viruses.  
Solid Waste: Autoclave 60 min. at 121°C.  
Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. The Substrate B contains 20% acetone, keep reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

## SAMPLE PREPARATION

### COLLECTION, HANDLING, AND STORAGE

- a) **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
- b) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulate. *This Syphilis ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.*
  - Avoid grossly hemolytic, lipidic or turbid samples.
  - Serum, plasma samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
  - When performing the assay slowly bring samples to room temperature.
  - It is recommended that all samples be assayed in duplicate.
  - DO NOT USE HEAT-TREATED SPECIMENS.

### PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below.

1. **Blank Control, Non-Reactive Control, and Reactive Control:** Supplied in prediluted form. DO NOT DILUTE.
2. **Wash Buffer:** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly by gently swirling before use. Avoid foaming. If a smaller volume of Wash Buffer is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer is stable for 1 month at 2-8°C. Mix well before use .
3. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

4. **Working Conjugate Solution:** First, bring Conjugate Diluent (Red coloured) to room temperature 10 to 30 minutes before use. Use the table provided to dilute Conjugate 1:100 with Conjugate Diluent before use in assay. Mix well.

Wells Used	Volume of Conjugate	Volume of Conjugate Diluent
16 wells	20 µL	1.98 mL
32 wells	40 µL	3.96 mL
48 wells	60 µL	5.94 mL
64 wells	80 µL	7.92 mL
80 wells	100 µL	9.90 mL
96 wells	120 µL	11.88 mL

## ASSAY PROCEDURE

1. Prepare Wash Buffer before starting assay procedure (see Preparation of Reagents). *It is recommended that the table provided be used as a reference for adding Controls and Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
<b>A1, B1</b>	Blank Control (BC)	<b>E1, F1, G1</b>	Reactive Control (RC)
<b>C1, D1</b>	Non-Reactive Control (NRC)	<b>H1.....</b>	Samples

2. Add 100 µL Blank Control, Non-Reactive Control, and Reactive Control to the appropriate wells of the Microtiter Plate. DO NOT DILUTE.
3. Prepare a (1:20) dilution of each sample by, first pipetting 95 µL of Sample Diluent into the appropriate Microtiter Plate wells, then adding 5 µL of sample. Mix well. Cover and incubate Microtiter Plate for 30 minutes at 37°C ± 2°C.
4. Prepare Working Conjugate Solution prior to washing the Microtiter Plate (see Preparation of Reagents).
5. Wash the Microtiter Plate using one of the specified methods indicated below:

**Manual Washing:** Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer, then aspirate contents of the plate into a sink or proper waste container.

Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350  $\mu\text{L}$ /well/wash (range: 350-400  $\mu\text{L}$ ). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

6. Add 100  $\mu\text{L}$  of Working Conjugate Solution to each well. Cover and incubate Microtiter Plate for 30 minutes at  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .
7. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
8. Repeat wash procedure as described in Step 5.
9. Add 100  $\mu\text{L}$  Substrate Solution to each well. Cover and incubate Microtiter Plate for 15 minutes at  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .
10. Add 50  $\mu\text{L}$  of Stop Solution to each well. Mix well.
11. Read the optical density (O.D.) at 450 nm of each well using microtiter plate reader within 30 minutes.

## QUALITY CONTROL

1. For each plate with each run of samples the Blank and Non-Reactive Control should be assayed in duplicate and the Reactive Control in triplicate.
2. Blank Control values must have an O.D. of  $\leq 0.15$
3. Non-Reactive Control values must have an O.D.  $\geq 0$  and  $\leq 0.15$  *after subtracting the Blank Control value.*
4. The Reactive Control must have at least two of the three values with an O.D.  $\geq 0.6$  and  $\leq 2.0$  *after subtracting the Blank Control value.*
5. Two or more of the Reactive Control values must not deviate by  $> 30\%$  from the control mean. If this occurs the run is not valid and the assay procedure must be repeated.
6. For the assay to be valid, the mean O.D. difference between Reactive Control and Non-Reactive Control must be  $\geq 0.500$  ( $RC - NRC \geq 0.500$ ). If, not poor technique must be suspected.

## RESULTS

1. All O.D. values for Controls and Samples are subtracted by the O.D. value of the Blank Control before result interpretation.
2. If more than one plate is being assayed at the same time, each plate must be calculated and interpreted separately.
3. The presence or absence of antibody to *T. Pallidum* is determined by relating the O.D. of the samples to the CUT-OFF value

## CALCULATION OF RESULTS

### 1. Calculation of Blank Control Mean O.D. ( $\overline{BC}$ )

Example:	<b>Well No.</b>	<b>O.D</b>	
	A1	0.022	
	B1	0.024	
	<i>Total</i>	<u>0.046</u>	
	<i>Mean</i>	0.046/2	= 0.023 ( $\overline{BC}$ )

### 2. Calculation of Non-Reactive Control Mean O.D. ( $\overline{NRC}$ )

Example:	<b>Well No.</b>	<b>O.D</b>	<b>BC subtracted</b>	
	C1	0.081	0.058	
	D1	0.083	0.060	
	<i>Total</i>		<u>0.118</u>	
	<i>Mean</i>		0.118/2	= 0.059 ( $\overline{NRC}$ )

### 3. Calculation of Reactive Control Mean O.D. ( $\overline{RC}$ )

Example:	<b>Well No.</b>	<b>O.D</b>	<b>BC subtracted</b>	
	E1	1.228	1.205	
	F1	1.273	1.250	
	G1	1.324	1.301	
	<i>Total</i>		<u>3.756</u>	
	<i>Mean</i>		3.756/3	= 1.252 ( $\overline{RC}$ )

### 4. Calculation of Reactive Control Mean - Non-Reactive Control Mean ( $\overline{RC} - \overline{NRC}$ )

Example:	$\overline{RC}$	=	1.252
	$\overline{NRC}$	=	0.059
	$\overline{RC} - \overline{NRC}$	=	1.252 - 0.059
		=	1.193

### 5. Calculation of CUT-OFF Value

$$\text{CUT-OFF value} = \overline{NRC} + 0.20$$

Example:	$\overline{NRC}$	=	0.059
	CUT-OFF value	=	0.059 + 0.20
		=	0.259

## **INTERPRETATION OF RESULTS**

1. Samples with O.D. values LESS THAN the Cut-off Value are considered NON-REACTIVE by the criteria of this Syphilis ELISA Kit. Further testing is not required.
2. Samples with O.D. values GREATER THAN or EQUAL to the Cut-off Value are considered INITIALLY REACTIVE for antibodies to T. pallidum by the criteria of this Syphilis ELISA Kit and should be re-tested in duplicate before final interpretation.
3. Samples that are found reactive on re-testing are interpreted to be REPEATEDLY REACTIVE for antibodies to T. pallidum by the criteria of this Syphilis ELISA Kit.
4. Initially reactive samples, which are found to be non-reactive on re-testing, are considered NEGATIVE for antibodies to T. pallidum by the criteria of this Syphilis ELISA Kit. Further testing is not required.