**SYphilis Total Antibody EIA**

**Cat. No.** 60125  Syphilis EIA 96 tests  
60126  Syphilis EIA 480 tests

**Kits for the qualitative and semi-quantitative detection of antibody to Treponema pallidum in human serum or plasma by enzyme-linked immunoassay.**

**CLINICAL BACKGROUND**

Syphilis is a chronic infection that progresses through distinct stages of infection: primary, secondary, tertiary, and quaternary. These stages produce diverse clinical symptoms, typically producing initial sores known as chancres then syphilitic rash followed by long periods of dormancy. Untreated infection may eventually result in cardiovascular problems and neurosyphilis.

The infection is caused by the spirochaete Treponema pallidum, and is usually acquired by sexual contact, although the disease may be transmitted by transfusion of infected blood. Intrauterine infection also occurs. The organism has proved virtually impossible to culture in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

Tests for syphilis fall into four categories: direct microscopic examination; treponemal antibody tests; non-treponemal antibody tests; and direct antigen tests. Because of the long periods of dormancy and the non-specific nature of non-treponemal tests, methods that detect specific anti-treponemal antibodies in blood specimens have become increasingly popular for screening. Lab21 Syphilis EIA is one such test.

**INTENDED USE**

These kits are intended for use by appropriately trained and qualified personnel for the detection of antibodies to Treponema pallidum in human serum and plasma. The product may be used for the screening of blood donors, the screening of pregnant women, and to aid in the diagnosis of patients where syphilis infection is suspected.

**PRINCIPLE OF THE TEST**

**SYPHILIS EIA 96 and 480 test kits** use three recombinant antigens in a sandwich test to produce a test that is both highly specific and sensitive. The antigens will detect T. pallidum-specific IgG, IgM, and IgA, enabling the test to detect antibodies during all stages of infection. All reagents except the Wash solution are supplied ready to use and colour-coded, and the procedure uses undiluted samples and standard volumes for ease of both manual and automated use. The assay can be used with both serum and plasma.

The plastic wells are coated with a mixture of the 15Kda, 17Kda, and 47Kda recombinant antigens of T. pallidum. Specific antibodies in serum or plasma specimens combine with these antigens and with the same antigens conjugated to horseradish peroxidase, when conjugate is added to a well in which the specimen has been incubated. After unreacted material has been removed by washing, the presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a colour change in the substrate/chromogen mixture. The intensity of the colour is compared to that in control wells to determine the presence or absence of specific antibody.

**KIT CONTENTS**

<table>
<thead>
<tr>
<th>Reagent Name</th>
<th>Description</th>
<th>Colour</th>
<th>60125 96 tests</th>
<th>60126 480 tests</th>
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<tbody>
<tr>
<td>R1</td>
<td>Plate</td>
<td></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>R2</td>
<td>Positive control</td>
<td>Human serum</td>
<td>Red</td>
<td>1.5mL</td>
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<tr>
<td>R3</td>
<td>Negative control</td>
<td>Human serum</td>
<td>Yellow</td>
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<tr>
<td>R4</td>
<td>Conjugate (Ready to use)</td>
<td>Recombinant antigens Conjugated to horseradish peroxidase</td>
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<tr>
<td>R5</td>
<td>Substrate</td>
<td>Urea peroxide and tetramethy benzidine</td>
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<tr>
<td>R6</td>
<td>Wash (20 x concentrated)</td>
<td>Saline containing surfactant</td>
<td>Colourless</td>
<td>125μL</td>
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<tr>
<td>R7</td>
<td>Stop</td>
<td>0.5M H2SO4</td>
<td>Colourless</td>
<td>7mL</td>
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</tbody>
</table>

**WARRANTS AND PRECAUTIONS**

**For in-vitro diagnostic use only.**

The control materials supplied are derived from human serum. They have been tested at donor level and found negative for Hepatitis B and C, and for HIV 1 and 2. However, they should be treated as if capable of transmitting disease.

Specimens of human serum and plasma should be treated as microbiologically hazardous, and handled in accordance with the applicable regulations.

Do not use the kit after its expiry date.

Do not combine or interchange reagents from kits with different lot numbers.

**STORAGE**

Store at 2-8°C when not in use. Store bottles upright.

**Do not freeze.**

**Do not expose substrate to direct sunlight.**

Diluted wash buffer is stable for 4 weeks at 4°C.

Unused coated strips are stable for 4 weeks at 4°C if stored in the re-sealable bag provided.

**EQUIPMENT REQUIRED**

- Properly calibrated and maintained pipetting devices capable of delivering volumes of 50μL (specimens and reagents) and approx 300μL (wash fluids)
- Plate or strip reader to read at 450nm and (optionally) at a wavelength between 620 and 690 nm
- 37°C incubator

Lab21 Syphilis EIA may be automated for both liquid handling and result interpretation. A variety of systems have been used for this – please consult the manufacturers of both the kit and the automation system for advice on automation.

Equipment should be able to support the following tolerances:

- Volume dispensed: +/- 10%
- Incubation temperature: +/- 2°C
- Incubation time: +/- 2 minutes

**SPECIMENS**

- Serum or plasma (collected into EDTA, sodium citrate, or heparin) specimens should be free of blood cells and of obvious microbial contamination. They may be stored at 2-8°C for up to 7 days before testing. Specimens needing longer storage should be frozen at -20°C or lower. Frozen specimens should be thawed and well mixed before testing. Samples have been validated up to 4 freeze/thaw cycles.

**ASSAY PROTOCOL**

**DMANUALE**

Bring all reagents and specimens to room temperature prior to use. Dilute Wash Buffer 1 in 20 with distilled or deionised water prior to use.

**Assay controls**

The Negative control must be tested three times with each lot of tests, and the Positive control twice.

**Verification of Reagent Addition**

**Visually:** Sample and negative controls are yellow

Plasma/sera + conjugate are green/blue

Positive control + conjugate are dark blue

Negative control + conjugate are green

**Automatic Reading:**

**Addition of the samples and controls** is verified by reading at 450/620nm. A well with sample added will have an A450-620 reading of between 0.050 and 1.00.

**Addition of conjugate** is verified by reading at 620/450nm. A well with sample and conjugate will have a value of ≥0.000, a well with sample only will have a value of <0.000.

**Addition of substrate** is verified by reading at 550nm. A well with substrate added must have an A550 greater than 0.080.

**Procedural Notes**

Washing must be thorough, with complete filling and emptying of the wells at each cycle.
Procedure
1. Add 50μL of the undiluted sample (or control – see "Assay Controls") to a coated well.
2. Add 50μL of conjugate to each well. The conjugate is supplied at working strength.
   Mix on a plate shaker for 30 seconds.
3. Incubate (covered) at 37°C for 30 minutes.
4. Wash 5x with working strength wash buffer. A short soak time of about 30 seconds is recommended between each wash cycle. Tap out excess liquid.
5. Add 50μL substrate/chromogen mixture to each well.
6. Incubate at room temperature for 30 minutes. As the substrate is photosensitive, it is recommended that the plate be protected from light during this incubation.
7. Add 50μL Stop to each well (blue colour changes to yellow)
8. Read results at 450nm (A450). Use of a reference filter at 620-690nm will eliminate effects of scratches, bubbles etc.

Cut-Off Value
Calculated as the mean of the negative control values plus 0.100 i.e.
\[ \frac{3}{\text{Example: } 0.030 + 0.025 + 0.035 = 0.030} \] 
\[ \text{Cut-Off Value} = 0.030 + 0.100 = 0.130 \]

ASSAY VALIDATION
A450 of each Negative Control should be lower or equal to 0.080. If one control is above this value the reading should be ignored and the cut-off calculated using the remaining two.
A450 of each Positive Control should be greater than or equal to 1.000

INTERPRETATION
Samples with A450 values less than the Cut-Off Value are considered negative by Syphilis EIA.
Samples with A450 values greater than or equal to the Cut-Off Value are considered positive by Syphilis EIA and should be re-tested in duplicate before final interpretation.
Re-tested samples that are above the cut-off in at least one duplicate are considered positive and should be investigated further. Samples that are below the cut-off in both duplicates are considered to be negative.

PERFORMANCE CHARACTERISTICS
Specificity
An independent study on 528 random donor samples showed specificity of 99.4% (95% confidence limits 97.44-100%). An external study on 40 normal serum samples from antenatal women showed specificity of 100% (95% confidence limits 98-100%).
No positive reactions were noted with a selection of samples from patients with the following diagnoses:
- Rheumatoid disease
- EB Virus
- Lyme Disease
- Leptospirosis
- SLE

Sensitivity
An internal study on 110 serum and plasma specimens from known cases of syphilis showed 100% sensitivity (95% confidence limits 98.02-100%).
An external study on 200 specimens representing all stages of syphilis showed 100% sensitivity (95% confidence limits 98.04-100%).
Of these, 25 specimens were of primary syphilis; all were positive showing 100% sensitivity (95% confidence limits 97.94-100%).

Analytical sensitivity
This kit has been shown to be capable of detecting between 0.0016IU and 49IU of antitreponemal antibody by testing dilutions of the First International Standard for SYPHILITIC SERUM, HUMAN: available from the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK. Over this range no high dose hook was evident.

BIBLIOGRAPHY

KEY TO SYMBOLS
[Diagram of symbols]
- In vitro diagnostic medical device
- Manufacturer
- Temperature limitation
- Use by
- Batch code
- Consult instructions for use

PRECISION

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<tr>
<th>Specimen No.</th>
<th>No. of replicates</th>
<th>Mean Auo/Auo</th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
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</thead>
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<td>(Negative)</td>
<td>20</td>
<td>0.013</td>
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</table>

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(ES) • Puede solicitar otros idiomas a su www.lab21.com/diagnosticproducts. Utilice obligatoriamente el folleto adjunto, versión indicada en la caja.


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