DRG® Syphilis (Treponema pallidum) ELISA (EIA-5218)

As of 10 Feb. 2011 rm (Vers. 1.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The kit is designed for the detection of the antibodies to *Treponema pallidum* (*T.pallidum*) in human serum or plasma by enzyme immunoassay.

2 PRINCIPLE OF THE ASSAY

The Syphilis test (EIA-5218) is a double sandwich ELISA for the detection of antibodies to *T.pallidum*. *T.pallidum*-specific antibodies contained in the test sample bind to the recombinant *T.pallidum* antigens (TP-47KDa and other antigens) coated on the microplate, and further bind to the enzyme conjugate to form an antigen/antibodies/antigen-enzyme complex. The wells are washed to remove unbound material. The enzyme substrate solution containing tetramethylbenzidine (TMB) is added. During incubation, the solution will develop blue colour if specific antibodies are present in the sample. The enzyme reaction is stopped by the addition of sulphuric acid. The intensity of colour developed is read spectrophotometrically at 450 nm and is proportional to the amount of antibodies present in the sample.

3 KITS CONTENTS

1. **Coated Microplate**: 1 plate (96 tests)
   Twelve 8-well strips per plate. Each microplate well is coated with recombinant *T.pallidum* antigens.
2. **Conjugate**: 1 bottle (6.2 mL)
   Phosphate buffered saline containing goat serum, protein stabilizer and recombinant *T.pallidum* antigens conjugated with horseradish peroxidase. Contains Proclin-300 as preservative.
3. **Positive Control**: 1 vial (1 mL)
   Diluted heat inactivated human serum containing antibodies to *T.pallidum*. Negative for HBsAg and anti-HCV, anti-HIV-1/2. Contains Proclin-300 as preservative.
4. **Negative Control**: 1 vial (1 mL)
   Diluted normal human serum, negative for HBsAg and anti-HCV, anti-HIV-1/2, *T.pallidum*.
   Contains Proclin-300 as preservative.
5. **Wash Solution Concentrate** (25x): 1 bottle (40 mL)
6. **Chromogen A** (Substrate solution): 1 bottle (8 mL)
   Citrate buffer containing hydrogen peroxide.
7. **Chromogen B** (Chromogen): 1 bottle (8 mL)
   3,3',5,5'-Teramethylbenzidine (TMB) dissolved in dimethylsulphoxide (DMSO).

8. **Stopping Solution:** 1 bottle (7 mL)
   2 M sulphuric acid solution.
9. **Adhesive Seals**
   Cover plate during incubation.
10. **Resealable Bag**
    For storage of unused strips.

4 MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled water
2. Manual or automatic pipettors capable of delivering 20 µL, 50 µL, 100 µL and 1000 µL.
3. Disposable pipette tips.
4. Timer
5. Microplate mixer
6. Incubator (37 °C)
7. Microplate reader (equipped with a 450 nm and 630 nm filter)
8. Gloves

5 STORAGE AND STABILITY

All of the kit components are stable until the expiration date shown on the label when stored at 2 °C – 8 °C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Unused strips should be stored at 2 °C – 8 °C, tightly sealed in the plastic bag provided with the desiccant inside. Once diluted, the washing solution is stable for one week at room temperature. The working substrate solution should be used once prepared.

6 SAFETY PRECAUTIONS

Syphilis test (EIA-5218) is a kit for research use only.

Handle samples, positive and negative controls as potentially infectious agents. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
Autoclave all used and contaminated materials at 121 °C, 15 psi for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes.
Wipe any spills promptly with 1% sodium hypochlorite solution.
The stopping solution is strong acid. Wipe spills immediately. Flush the area of the spills with water. If the stopping solution contacts the skin or eyes, flush with copious amounts of water and seek medical attention.

7 HANDLING INSTRUCTION:

Do not use the kit after the expiration date.

Do not substitute reagents from one kit lot to another.
Do not use mouth to pipette.
Use only reagent grade quality, deionized or distilled water to dilute reagents.
Do not expose substrate and TMB to strong light.
Avoid contact of TMB and sulphuric acid with any oxidizing agent or metal.
Avoid repeatedly opening and closing the incubator during incubation steps.

8 PREPARATION OF REAGENTS

1. Washing solution

The washing solution (1X) is a 1:25 dilution of the washing solution concentrate (25X) provided with the kit.

Prepare washing solution (1X) as needed by adding one part concentrated solution (25X) to twenty-four parts deionized or distilled water. The diluted washing solution (1X) can be stored at room temperature for up to one week.

Note: Crystals may form when washing solution concentrate is stored at 2 °C – 8 °C. This must be dissolved by warming to 37 °C prior to use.

2. Working substrate solution

The working substrate solution is a 1:1 combination of the substrate solution (Chormogen A) with the chromogen (Chomogen B).

For every two strips to be tested, add 1 mL of the chromogen (Chomogen B) to 1 mL of the substrate solution (Chormogen A) as shown in the following table.

Number of strips to be used	2	4	6	8	10	12
Amount of substrate solution (Chormogen A)	1	2	3	4	5	6
Amount of chromogen (Chomogen B)	1	2	3	4	5	6

Note:

1. *Do not mix all of the substrate solution with the chromogen, extra reagents are provided.*
2. *It is recommended that the working substrate solution should be used in 20 minutes.*
3. *The working substrate solution should be colorless. A distinct blue color indicates that solution is contaminated. Discard the working substrate solution and prepare fresh solution in a clean container.*

9 PROCEDURE

1. Bring all reagents to room temperature before the beginning of the assay procedure.
2. Remove microplate from the aluminium bag; put unused strips and desiccant into the resealable bag, store at 2 °C – 8 °C.
3. Add **50 µL** of Negative Control to each of the two wells; use a clean pipette tip for addition.

4. Add **50 µL** of Positive Control to each of the two wells; use a clean pipette tip for addition.
5. Pipette **50 µL** of specimen to the assigned wells.
   Then add **50 µL** of Conjugate to these wells.
   In every test leave two wells as blank and do not add specimen or conjugate to the blank wells.
6. Cover the plate with adhesive seal, mix the plate and incubate for 30 minutes at 37 °C.
7. During the last 5 minutes of this incubation, prepare working substrate solution as described above.
8. Remove and discard the adhesive seal. Aspirate the content of the wells and wash the plate five times with diluted washing solution. Aspirate the washing solution each times, after the last wash, blot the inverted plate on absorbent paper towels to remove any excess liquid from the wells.
9. Add **100 µL** of the working substrate solution to each well.
10. Incubate for 10 minutes at 37 °C.
11. Add **50 µL** of stopping solution to each well to terminate the reaction.
12. Read the absorbance for each well at 450 nm. If a dual filter instrument is used, the reference wavelength should be 630 nm.

Note:

1. *Once the assay has been started, it should be completed without interruption.*
2. *Absorbance should be read within 1 hour of the addition of the Stopping Solution.*
3. *Do not use the microplate washer to aspirate acid and do not aspirate acid into bleach.*

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10 REFERENCES / LITERATURE

1. Farnes SW and Setness P: Serologic Tests for Syphilis. Post Graduate Medicine. 87(3): 37-46, 1990.
2. Farshy CE, Hunter EF, Larsen SA and Cerny EH: Double Conjugate Enzyme-linked Immunosorbent Assay for Immunoglobulins G and M against Treponema Pallidum Antibody. Journal of Clinical Microbiology. 20(6): 1109-1113, 1984.
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5. Lennette DA: Collection and preparation of specimens for virological examination. In: Manual of Clinical Microbiology, 4th edition. Lennette EH, Balows A, Hausler WJ, Shadomy HJ, Eds American Society for Microbiology, Washington DC.Ch.61, pp 687-693, 1985.