DRG® Borrelia burgdorferi IgM ELISA (EIA-4485)

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REVISED 15 JULY 2013 RM (VERS. 3.1) **RUO IN THE USA**

This kit is intended for Research Use Only

Not for Diagnostic Purposes

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

1 INTENDED USE

The Anti-Borrelia IgM is for measurement of IgM antibodies against *Borrelia burgdorferi* in human serum or plasma samples.

2 PRINCIPLE OF THE TEST

The *Anti-Borrelia* IgM kit is for tmeasurement of IgM antibodies against a mixture of antigens from *Borrelia afzelii* and *Borrelia garinii* supplemented with VsE in human serum.

The antibodies of the positive control and diluted specimen samples react with immobilized antigens on the solid phase of microtitration plates. Following an incubation period of 30 min at 37 °C, unbound serum components are removed by washing the wells three times with wash buffer.

The bound antibodies react specifically with anti-human-IgM conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at 37 °C. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colourless substrate solution of 3,3',5,5'-tetra-methylbenzidine (TMB) / hydrogen peroxide added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (sulphuric acid) into the wells after 15 min at 37 °C turning the solution from blue to yellow.

The absorbances read at 450/≥620 nm are directly proportional to the concentration of specifically bound antibodies.

3 PREPARATION AND STORAGE OF SAMPLES

3.1 Collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, haemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

3.2 Preparation before use

Allow samples to reach RT prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Specimen samples have to be diluted 1 : 101, e.g. 10 µL sample + 1000 µL sample diluent *(3)*, prior to assay.

The samples may be kept at 2 °C - 8 °C for up to two days. Long-term storage requires -20 °C.
(The controls are ready-to-use and should not be diluted).

4 TEST COMPONENTS FOR 96 DETERMINATIONS

1 **WELLS**	**Microtitration plate,** 12 breakable 8 wells strips (total 96 individual wells) coated with antigens from *Borrelia afzelii, Borrelia garinii* and VlSE.	1 vacuum sealed with desiccant
2 **WASHBUF CONC 10X**	**Concentrated wash buffer** for 1000 mL solution	100 mL concentrate white cap
3 **DIL**	**Sample diluent**	100 mL ready to use black cap coloured red
4 **CONTROL +**	**Positive Control** (diluted serum) Concentration see leaflet enclosed	1.0 mL ready to use red cap coloured blue
5 **CONTROL –**	**Negative Control** diluted serum	1.0 mL ready to use green cap coloured blue
6 **CONJ HRP**	**Conjugate** Containing anti-human-IgM (sheep) coupled with HRP	15 mL ready to use red cap coloured red

7 **SUBSTR TMB**	**Substrate** 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide	15 mL ready to use blue cap
9 **STOP**	**Stop solution** 0.25 M sulphuric acid	15 mL ready to use yellow cap

5 MATERIALS REQUIRED BUT NOT PROVIDED

- Adjustable one channel micropipette 0.100 – 1.000 mL and 010 – 0.100 mL
- Adjustable 8-channel micropipette 0.050 – 0.200 mL - Pipette tips
- Graduated measuring flasks 10 mL and 100 mL
- Microtitration plate washer (automatic or hand wash head)
- Microtitration plate reader with 450 nm filter for measurement and ≥ 620 nm filter for reference
- Distilled or de-ionized water
- Test tubes (2 mL) for sample dilution

6 PREPARATION AND STORAGE OF REAGENTS

6.1 Kit size and expiry

The *Anti-Borrelia* IgM has been designed for 96 determinations.

The complete kit with unopened reagent bottles and microtitration strips is stable until the expiry date printed on the kit box in case of storage at 2 °C - 8 °C.

Once opened all kit components are stable for up to 2 months under appropriate storage conditions (2 °C - 8 °C). When stored at 2 °C - 8 °C the diluted ready-to-use wash solution is stable for up to 1 month.

6.2 Reagent preparation

Allow all components to reach RT prior to use in the assay.

The microtitration plate is vacuum sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the **WASHBUF CONC 10X** ***(2)*** 10 times (1 + 9) with deionized or distilled water.

For example: 10 mL **WASHBUF CONC 10X** ***(2)*** + 90 mL distilled water.

Avoid exposure of the TMB substrate solution to light!

7 ASSAY PROCEDURE

- Dilute specimen sera with sample diluent (3) **1 + 100** (v/v)
  e.g. 10 µL serum + 1000 µL sample diluent (3)
- Avoid any time shift during dispensing of reagents and samples.
- Make sure the soaking time of the wash solution in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every wash cycle.

7.1 Working steps

1. Warm all reagents to RT before use. Mix gently without causing foam.
    1. Dispense
       100 µL *CONTROL+* (ready-to-use control) ***(4)***
       100 µL *CONTROL-* (ready-to-use control) ***(5)*** and
       100 µL diluted serum samples resp
       into the intended wells.
    2. Cover plate, incubate **30 min** at 37°C.
    3. Decant, then wash wells **three** times using **300 µL** wash solution (made of ***(2)***).
    4. Add **100 µL *CONJ HRP (6)*** to each well.
    5. Cover plate, incubate **30 min** at 37°C.
    6. Decant, then wash wells **three** times using **300 µL** wash solution (made of ***(2)***).
    7. Add **100 µL *SUBSTR TMB (7)*** to each well.
    8. Incubate **15 min** at 37°C protected from light.
    9. Add **100 µL *STOP (8)*** to each well and mix gently.
       Read absorbances at **450 nm / ≥ 620 nm** within 30 min after reaction stop.

8 RESULT INTERPRETATION

Calculation of the cut off and borderline zone

The cut off value is calculated as:

Cut off = mean value of the absorbance of the negative control (5) plus 0.4 absorbance units

The borderline area is calculated as:

Borderline zone = (0.9 x cut off) until cut off value

8.1 Limitations of the procedure

Reactive serum samples should be verified by a confirmatory test (e.g. Line-Assay or Westernblot).

As in other immunoassays, impurities and cross contamination of reagents and samples by fungi and bacteria can produce false results.

9 COMMON ADVICES AND PRECAUTIONS

Follow the working instructions carefully. The kit should be performed by trained technical staff only.

The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

Do not use or mix reagents from different lots or reagents from other manufacturers.

Avoid time shift during dispensing of reagents.

All reagents should be kept at 2 °C - 8 °C before use.

Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.

Handle all components and all specimen samples as if potentially hazardous.

Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.

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