DRG® Borrelia burgdorferi IgG ELISA (EIA-4486)

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REVISED 15 MAY 2008 RM (VERS. 1.1) **RUO IN THE USA**

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTENDED USE

The Borrelia burgdorferi-IgG ELISA is a device for measurement of human anti-Borrelia burgdorferi-IgG-Antibodies in serum, plasma or cerebrospinal fluid.

2 PRINCIPLE OF THE TEST

The ***-Borrelia burgdorferi* IgG ELISA** is an immune enzymometric two step assay based on an insolubilized antigen mixture of a *Borrelia afzelii* strain **additionally supplemented with the highly specific VlsE antigen**.

Samples as well as calibrators are pipetted into the microwells that are coated with the antigen. After 30 minutes of incubation at 37 °C, unbound components are removed and the wells are washed 3 times with wash buffer. After addition of anti-human-IgG-F(ab)$_2$-HRP conjugate, wells are incubated for 30 minutes at 37 °C, unbound components are then removed and washed 3 times with wash buffer. After that ready-to-use TMB/substrate solution (tetramethylbenzidine and hydrogen peroxide) is added to the wells. The incubation time is 15 minutes at room temperature and the reaction is stopped by addition of sulphuric acid to the wells. Absorbances are read with a microplate reader at 450 nm wavelength (reference filter 620 nm wavelength if possible). Results are interpreted in referrence to the absorbances of the calibrators.

3 PREPARATION AND STORAGE OF SAMPLES

Serum, plasma or cerebrospinal fluid can be investigated for anti *Borrelia burgdorferi* IgG antibodies with the *Borrelia burgdorferi* IgG ELISA.

Serum or plasma samples have to be diluted **1:101** with sample diluent,
e. g. 5 µl sample + 500 µl sample diluent.

Pay attention to a sterile sample collection. Samples can be stored at 2 - 8 °C for a maximum time of 48 hours. For longer storage times samples have to be stored at - 20 °C.

Frozen samples have to be warmed to room temperature slowly and mixed well before starting the test run. Repeated freezing and thawing of samples should be avoided.

4 TEST COMPONENTS FOR 96 WELLS

Microtiter plate	**12 single breakable 8-well strips (96 wells) coated with purified *Borrelia afzelii* antigen containing additionally a VlsE supplement**	**1 vacuum-sealed with desiccant**
Wash buffer, 10X	10-fold; for 1000 ml solution	1 x 100 ml concentrate white cap
Sample Diluent		100 ml ready to use black cap
Standard 1 - 4	Std. 1 = 40 U/ml Std. 2 = 100 U/ml Std. 3 = 250 U/ml Std. 4 = 1000 U/ml	1 ml each, ready to use, white cap
Neagtive Control	Diluted serum	1 ml, ready to use, green cap
Positive Control	Containing anti Borrelia IgG antibodies. See QC data sheet	1 ml, ready to use, red cap
HRP Conjugate	HRP-labelled, polyclonal anti-bodies (sheep)	15 ml ready to use red cap
Substrate Solution	Substrate 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml ready to use blue cap
Stop Solution	Stop solution 0.25 M sulphuric acid	15 ml ready to use yellow cap

5 MATERIALS REQUIRED BUT NOT PROVIDED

- micropipettes
- multi-channel pipette or multi-pipette
- glassware
- tubes (1 ml) for sample preparation
- pipette tips
- Reagent container for multi-channel pipette

- Incubator (37 °C)
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

6 PREPARATION AND STORAGE OF REAGENTS

6.1 Kit size and expiry

One kit is designed for 96 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label.

Upon receipt, all test components have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 4 weeks, provided proper storage.

6.2 Reagent preparation

Allow all components to reach room temperature 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. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Washing solution

Prepare a sufficient amount of wash solution by diluting the 10fold concentrated wash buffer **1 + 9** with distilled or de-ionized water.

For Example:

10 ml wash buffer concentrate + 90 ml distilled water.

This ready to use wash buffer solution is stable for at least 30 days when stored at 2 - 8 °C.

Make sure that the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!

Avoid light exposure of the TMB substrate solution!

7 ASSAY PROCEDURE

- **Dilute samples with Sample Diluent 1 + 100,**
  e.g. 5 µl serum + 0.5 ml sample diluent
- **Avoid any time shift during dispensing of reagents and samples.**

7.1 Working steps

1. Warm all reagents to room temperature before use. Mix gently without foaming.
2. Dispense **100 µl of each Standard, 100 µl of each Control** and **100 µl of the diluted sample**
3. Cover plate and incubate for 30 min at 37°C.
4. Aspirate, then wash each well **3x** with **300 µl** diluted wash solution and tap dry onto absorbent paper.
5. Dispense **100 µl HRP-Conjugate**.
6. Cover plate and incubate for 30 min at 37°C.
7. Aspirate, then wash each well **3x** with **300 µl** diluted wash solution and tap dry onto absorbent paper.
8. Dispense **100 µl Substrate Solution**.
9. Incubate for 15 min at room temperature protected from light.
10. Dispense **100 µl Stop Solution** mix gently.
11. Read OD at 450 nm (reference filter 620 or 690 nm) with a microplate reader within 30 min after reaction stop.

8 RESULT INTERPRETATION

Create a reference curve by plotting the measured calibrator absorbances (y-axis) against the corresponding antibody concentrations of the standards (x-axis). Determine the antibody concentration of the sample in units/ml from the corresponding absorbances of the diluted samples.

Note that the standard concentrations already take into account the regular dilution of the samples (1:101)!

Samples with absorbances higher the Standard 4 should be retested in a higher predilution. This additional dilution factor has to be taken into consideration.

9 LIMITATIONS OF THE PROCEDURE

Serum samples reactive for Borrelia burgdorferi IgM and/or IgG antibodies in the ELISA should be verified with a confirmatory test like western blot (e. g. Serablot Human Anti-Borrelia burgdorferi IgG/IgM).

Microbial contaminations of reagents or samples as well as cross contaminations of test kit components and samples can cause false results.

Incorrect washing for separation of unbound sample or reagent components as well as incorrect incubation times can cause false results.

10 COMMON ADVICE AND PRECAUTIONS

This kit is for research use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only.
The expiry dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

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Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.
Do not use reagents from other manufacturers.
Avoid time shift during dispensing of reagents.
All reagents should be kept at 2 °C to 8 °C before use.
Some of the reagents contain small amounts of Thimerosal (< 0.01 % 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.