The NativeAntigen COMPANY

Instructions for Use

Borrelia burgdorferi IgG ELISA

Enzyme immunoassay for the detection and quantitative determination of human IgG antibodies against Borrelia burgdorferi in serum and plasma

1 x 96 well plate

ELS61233

Read the documentation

For Research Use Only

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1. INTENDED USE

The Borrelia burgdorferi IgG antibody ELISA kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Borrelia burgdorferi in serum and plasma.

2. GENERAL INFORMATION

Borrelia burgdorferi belongs to the family of spirochetes, of which three types have been identified to be pathogenic to humans: Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii. The endemic areas of Borrelia are in Central and Eastern Europe, as well as Russia, China and Japan. The illness is transferred via tick bites, in Europe mainly by Ixodes ricinus. In endemic zones, like Southern Germany and Austria, up to 50% of ticks are infected.

In the clinical course, after an erythema migrans, e.g. with neuroborreliosis, which appears at the first stage, chronical arthritis, encephalitis, meningitis, myositis and hepatitis are also observed. Treatment is usually by different antibiotics, e.g. doxycyclin, amoxicillin, cefuroxim and penicillin G. Specific immunization is possible with immunoprophylaxis either by a recombinant OspA or by a recombinant polyvalent OspC vaccine.

Laboratory diagnosis is performed by the detection of antibodies in blood and cerebrospinal fluid. Methods employed are ELISA, immunofluorescence, hemagglutination or Western blot. Besides whole cell extracts, purified or recombinant single proteins are increasingly used as antigens. This generally results however in a decrease in sensitivity. It has been shown that there are significant differences between the various test methods used for interpretation and the most reliable method seems to be the follow-up of titer development. Western blot serves as a confirmatory test, because electrophoretically separated single antigens can be evaluated in their reaction with specific serum antibodies.

3. PRINCIPLE OF THE TESTS

The Borrelia burgdorferi IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Borrelia burgdorferi antigen is bound on the surface of the microtiter strips. Diluted serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Borrelia burgdorferi antigen takes place. After one-hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at a wavelength of 450 nm. The concentration of IgG antibodies is directly proportional to the intensity of the color. The Borrelia burgdorferi IgG ELISA test kit contains both a whole cell antigen extract of Borrelia burgdorferi sensu stricto (which cross-reacts with Borrelia afzelii and Borrelia garinii) and pure OspC, which greatly increases the specificity and sensitivity of the assay.

4. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab should be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves must be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and must be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before using the kit.
- Before pipetting, all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking and formation of foam should be avoided.
- It is important to pipet at constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further, a possible mix-up should be avoided. The content of the bottles is usually sensitive to oxidation, so should be opened for only a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding their accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication (see MSDS).

5. REAGENTS PROVIDED

Reagent	Components	Volume / Qty.
SORB MT	Borrelia burgdorferi antigen coated microtiter strips	12
CAL A	Calibrator A (Negative Control)	2 mL
CAL B	Calibrator B (Cut-Off Standard)	2 mL
CAL C	Calibrator C (Weak Positive Control)	2 mL
CAL D	Calibrator D (Positive Control)	2 mL
ENZ CONJ	Enzyme Conjugate	15 mL
SUB TMB	Substrate	15 mL
STOP SOLN	Stop Solution	15 mL
SAM DIL	Sample Diluent	60 mL
WASH SOLN 10x	Washing Buffer (10×)	60 mL

Storage and Stability (refer to the expiry date on the outer box label)
Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with Borrelia antigen (mix of Borrelia sensu stricto, Borrelia afzelii and Borrelia garinii). Ready-to-use.

5.2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgG antibodies against Borrelia burgdorferi. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. Calibrator B (Cut-Off Standard)
2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Borrelia burgdorferi. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.4. Calibrator C (Weak Positive Control)
2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Borrelia burgdorferi. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.5. Calibrator D (Positive Control)
2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Borrelia burgdorferi. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.6. Enzyme Conjugate
15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

5.7. Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution
15 mL, 1 N acidic solution. Ready-to-use.

5.9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.10. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

6. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Deionized water
- Re-usable black lid for covering
- Plastic bag

7. SAMPLES TO BE TESTED

Principally serum or plasma (EDTA, heparin) can be used for the determination. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

8. ASSAY PROCEDURE

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the re-usable plate cover and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (pour or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated three times in total. Remaining washing buffer is removed afterwards by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the re-usable plate cover and incubate at room temperature for 30 minutes.

7. Empty the wells of the plate (pour or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated three times in total. Remaining washing buffer is removed afterwards by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time, the substrate blank is also pipetted.
9. Cover plate with the re-usable plate cover and incubate at room temperature for 20 minutes in the dark.
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The colour is stable for at least 60 minutes.

9. EVALUATION

Example:

	OD Value	Corrected OD
Substrate Blank	0.020	
Negative Control	0.032	0.012
Cut-Off Standard	0.522	0.502
Weak Positive Control	0.815	0.795
Positive Control	1.699	1.679

The above table contains an example achieved under arbitrary temperature and environmental conditions. The described data do not constitute reference values; these will be determined by the user.

9.1. Qualitative Evaluation

The calculated absorptions for the test sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It is reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case, repetition of the test with the same serum (or with serum from the same source), is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least double the absorption compared with the cut-off standard.

9.2. Quantitative Evaluation

The ready-to-use standards and controls of the Borrelia IgG antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. The values for controls and standards in units are printed on the QC Certificate. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn point-to-point against their concentrations. From the resulting reference curve, the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automated computer programs. As curve fit point-to-point has to be chosen. Calibrator B with its concentration of 10 U/mL serves as cut-off standard. Analogous to the qualitative evaluation, a range of +/-20% around the cut-off is defined as a grey zone. Thus, results between 8 and 12 U/mL are reported as borderline.

10. ASSAY CHARACTERISTICS

Borrelia burgdorferi ELISA	IgG
Intra-Assay-Precision	4.8 %
Inter-Assay- Precision	7.7 %
Inter-Lot- Precision	2.8 – 10.6 %
Analytical Sensitivity	0.99 U/mL
Recovery	79 – 88 %
Linearity	77 – 126 %
Cross-Reactivity	No cross-reactivity to TBEV (FSME)
Interferences	No interferences to bilirubin up to 0.3 mg/mL haemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.
Clinical Specificity	97 %
Clinical Sensitivity	88 %

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