

Borrelia burgdorferi

IgG – ELISA (recombinant)

Enzyme immunoassay for the qualitative determination of IgG-class antibodies against *Borrelia burgdorferi* in human serum or plasma

For laboratory use only.

Product Number: NB-06-0040 (96 Determinations)

1. INTRODUCTION

Spirochetes are motile bacteria with a periplasmic axial filament. All pathogenic species belong to the family Treponemataceae, which includes the three genera: Treponema, Borrelia, and Leptospira. The Treponemae are extremely long, flexible, filamentous cells that are usually held in a characteristic spiral, or coiled-spring shape. Borreliae are the largest Treponemataceae with very coarse and irregular spirals. Borrelia burgdorferi, the causative agent of Lyme disease, is transmitted mainly by ticks but probably also by other blood-sucking insects. Habitats are the wooded, humid and temperate regions of North America, Europe, North Africa, Australia and Japan; foresters, farmers, and anybody entering infested forests may be affected.

The degree of contamination of ticks amounts to 3-60% dependent on seasonal and regional differences; up to 30% of the population may be infected (about 1500 cases annually in USA, several hundred in Europe).

Species	Diseases	Symptoms	Mechanism of infection
Borrelia burgdorferi	Lyme diseases	Erythema chronicum migrans (ECM), Bannwarth Syndrome; dermatologic, rheumatic, cardiologic and neurologic: multisystem disorder	Transmission by tick bites infected with Borrelia Borrelia burgdorferi (Ixodes dammini, USA: Ixodes ricinus, Europe)

Because of its multisystemic character, unequivocal diagnosis of Lyme disease is difficult. Only early detection permits efficient control by antibiotics, since in the chronic phase borreliae are nearly inaccessible.

The presence of bacteria resp. infection may be identified by

- Microscopy: Giemsa or Wright-stained blood smears
- PCR
- Serology: Detection of antibodies by IF, ELISA, immunoblotting

2. INTENDED USE

The **recombinant Neo Biotech Borrelia burgdorferi IgG-ELISA** is intended for the qualitative determination of IgG class antibodies against Borrelia burgdorferi in human serum or plasma (citrate) offering increased diagnostic specificity and sensitivity by employing immunodominant antigens. In combination with the recombinant IgM ELISA each clinical phase of Lyme disease - from Erythema chronicum migrans over Bannwarth-Syndrom up to Lyme arthritis - may be detected even in borderland cases providing unerring results and diagnostic aid for the clinical staff.

3. PRINCIPLE OF THE ASSAY

The major constituent of B.burgdorferi flagella is flagellin (41 kDa, p41). Whereas the lipoprotein OspC (22 kDa, p22) within the outer membrane of the spirochete induces an early antibody formation in the ECM-phase of Lyme disease, the species-specific markers p100, p18 and VlsE are especially reliable for detecting the IgG response and they are responsible for the high sensitivity. P41i is included in the presented antigens to avoid cross reactivity with antibodies of syphilitic sera.

The Neo Biotech Borrelia burgdorferi IgG-ELISA contains the recombinant epitope OspC of the phylum B31 (B. sensu stricto), 20047 and T25 (B. garinii), p100, and p18 of the phylum PKo (B. afzelii) and p41i of the phylum PBi (B. garinii).

Microtiter strip wells are precoated with recombinant Borrelia burgdorferi antigens. Diluted patient specimens and ready to use controls are added to these wells and antibodies recognizing the immobilized B. burgdorferi antigen bind during the first incubation. After washing the wells to remove all unbound sample and control material horseradish peroxidase labelled anti-human IgG conjugate is added. During a second incubation this conjugate binds to the captured antibodies, and the excess unbound conjugate is removed by a further wash step. The immune complex formed by the bound conjugate is visualized with TMB Substrate Solution which gives a blue reaction product, the intensity of which is proportional to the amount of B. burgdorferi-specific IgG antibody in the patient specimen. Sulphuric acid is added to each well to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Borrelia burgdorferi Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with Borrelia burgdorferi antigen; in resealable aluminium foil.
- **IgG Sample Diluent ***:** 1 bottle containing 100 ml of ready to use buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; white cap.
- **Stop Solution:** 1 bottle containing 15 ml. Ready to use sulphuric acid, 0.2 mol/l; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle each containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Borrelia burgdorferi anti-IgG Conjugate**:** 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to human IgG.; coloured blue, Ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Borrelia burgdorferi IgG Positive Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
- **Borrelia burgdorferi IgG Cut-off Control***:** 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.

- **Borrelia burgdorferi IgG Negative Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.
- * contains 0.1 % Bronidox L after dilution
- ** contains 0.2 % Bronidox L
- *** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foil
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20...25°C) before starting the test run!

6.1. Coated snap-off strips

The ready to use breakapart snap-off strips are coated with *Borrelia burgdorferi* antigen. Store at 2...8°C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. Borrelia burgdorferi anti-IgG Conjugate

The bottle contains 20 ml of a solution with anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use. Store at 2...8°C. *After first opening stability expiry date when stored at 2...8°C.*

6.3. Controls

The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2...8°C.*

6.4. IgG Sample Diluent

The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. *After first opening stability expiry date when stored at 2...8°C.*

6.5. Washing solution (20xconc.)

The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. *After first opening the concentrate is stable until expiry date when stored at 2...8°C.*

6.6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability expiry date when stored at 2...8°C.*

6.7. Stop Solution

The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C. *After first opening stability until expiry date.*

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
1 well	(e.g. B1)	for the negative control,
2 wells	(e.g. C1+D1)	for the cut-off control and
1 well	(e.g. E1)	for the positive control.

It is left to the user to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl Borrelia burgdorferi anti IgG conjugate into all wells except for the blank well (e.g. A1). Cover with foil
6. **Incubate for 30 min at room temperature. Do not expose to direct sunlight.**
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB substrate.
Any blue colour developed during the incubation turns into yellow.
Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Assay Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value < **0.100**.
- **Negative control** in B1: Absorbance value < **0.200 and < cut-off**
- **Cut-off control** in C1 and D1: Absorbance value **0.150 – 1.30**.
- **Positive control** in E1: Absorbance value > **cut-off**.

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 = 0.76 / 2 = 0.38

$$\text{Cut-off} = 0.38$$

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative
→ **grey zone**

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in Neo Biotech Units

$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Neo Biotech-U nits} = \text{NTU}]$

Example: $\frac{1.216 \times 10}{0.38} = 32 \text{ NTU (Neo Biotech Units)}$

Cut-off:	10	NTU
Grey zone:	9-11	NTU
Negative:	<9	NTU
Positive:	>11	NTU

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

Intraassay	n	Mean value	CV (%)
Pos. Serum	7	1.84	4.6
Interassay	n	Mean value	CV (%)
Pos. Serum	3	1.61	4.0

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100 %.

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 98.6 %.

10.4. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunosuppressed patients and newborns serological data only have restricted value.

A neg. result (IgG or IgM) cannot exclude an infection with *B. burgdorferi*. Especially in the early phase of infection there is the possibility that none or no detection quantities of the antibodies do exist. In the case of infection or a grey zone result we recommend a further sample after 2-3 weeks. A positive result of IgG does not always mean that an acute infection exists because the antibodies of a previous infection can persist. The use of recombinant antigens avoid extensively cross reactions with the following antibodies: *Trponema pallidum*, *Leptospira*, *Borrelia recurrentis*. Antibodies of a lues-infection to p41i were occasionally determined. Therefore a lues-infection should be excluded.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The Neo Biotech ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
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WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!
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12.1. Disposal Considerations






Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Product Number: NB-06-0040 *Borrelia burgdorferi* IgG-ELISA (96 Determinations)

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Symbols Key	
	Manufactured by
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date
	Storage Temperature
CE	CE Mark
[REF]	Catalogue Number
	Consult Instructions for Use
MTP	Microplate
CONJ	Conjugate
CONTROL -	Control serum, negative
CONTROL +	Control serum, positive
CUT OFF	Cut off control serum
DIL G	Sample diluent buffer IgG
SOLN STOP	Stop solution
SUB TMB	TMB Substrate solution
WASHBUF 20x	Washing solution 20x concentrated
	Contains sufficient for “n” tests

SCHEME OF THE ASSAY

Borrelia burgdorferi IgG-ELISA

Assay preparation

Prepare reagents and samples as described.
 Establish the distribution and identification plan for all specimens and controls on the form supplied in the kit.
 Select the required number of microtiter strips or wells and insert them into the holder.

Assay procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut-off control	Sample (diluted 1+100)
Negative control	-	100µl	-	-	-
Positive control	-	-	100µl	-	-
Cut-off control	-	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	-	100µl
Cover wells with foil supplied in the kit Incubate for 1 h at 37°C Wash each well three times with 300µl of washing solution					
Conjugate	-	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit Incubate for 30 min at room temperature Wash each well three times with 300µl of washing solution					
TMB Substrate	100µl	100µl	100µl	100µl	100µl
Incubate for exactly 15 min at room temperature in the dark					
Stop Solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					