DRG® Borrelia 14kD and OspC IgM ELISA (EIA-4288)

CE

Revised 25 July 2012 RM (Vers. 7.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

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

INTENDED USE

Enzyme immunoassay for measurement of IgM antibodies against the 14 kDa and OspC antigens of Borrelia burgdorferi in human serum, plasma and CSF.
Infections with all three B. burgdorferi subspecies (garinii, afzelii and senso strictu) are detected

TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgM. After the substrate reaction the intensity of the color developed is proportional to the amount of IgM-specific antibodies detected. Results of samples can be determined directly using the standard curve or calibrator.

WARNINGS AND PRECAUTIONS

1. For Research use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact DRG or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available upon request.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2 °C - 8 °C. Keep away from heat or direct sun light.
The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to 3 months after the first opening when stored at 2 °C - 8 °C in the tightly closed bag.

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SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly haemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage Serum/Plasma/CSF:	2 °C - 8 °C	≤ -20 °C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability Serum/Plasma/CSF:	5 days	12 months	

MATERIALS SUPPLIED

Quantity	Symbols	Component
1 x 12 x 8	MTP IgM	**Microtiter Plate** Break apart strips. Coated with specific antigen.
1 x 12 mL	ENZCONJ IgM	**Enzyme Conjugate** Ready to use. Red colored. Contains: anti-human IgM, conjugated to peroxidase.
1 x 4 x 1.5 mL	CAL A-D	**Standard A-D** 2; 10; 25; 100 U/mL Standard B = Calibrator Ready to use. Contains: IgM antibodies against B. burgdorferi, stabilizers.
1 x 1.5 mL	CONTROL +	**High Control** Ready to use. Contains: IgM antibodies against B. burgdorferi, stabilizers.
1 x 1.5 mL	CONTROL -	**Low Control** Ready to use. Contains: human serum, stabilizers.
1 x 100 mL	DILBUF M	**Diluent Buffer IgM** Ready to use. Blue colored. Contains: RF-Absorbent (goat anti-human IgG).
1 x 100 mL	WASHBUF CONC	**Wash Buffer, Concentrate (10x)** Contains: phosphate buffer.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 5; 10; 100; 1000 µL (adjustable)
2. Vortex mixer
3. Tubes (≥ 1 mL) for sample dilution
4. Incubator, 37 °C

5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18 °C - 25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

PRE-TEST SETUP INSTRUCTIONS

A. Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
100 mL	WASHBUF CONC	ad 1000 mL	bidist. water	1:10	Resolve crystals at 18-25°C.	2 °C - 8 °C	2 months

B. Dilution of Samples

Serum, Plasma

Sample	to be diluted	with	Relation	Remarks
Serum, Plasma	generally	DILBUF	1:101	e.g. 10 µL + 1 mL

Samples containing concentrations higher than the highest standard have to be diluted further.

Serum/CSF

For diagnostics of cerebrospinal fluid (CSF) according to Reiber, it is necessary to use approximately similar concentrations or Cut-off indices (COI) in the OD range of 2.0 to 0.3 for serum and CSF. This is generally ensured with the following dilutions:

Sample	to be diluted	with	Relation	Remarks
Serum	generally	DILBUF	1:401	e.g. 5 µL + 2 mL
CSF	generally	DILBUF	1:4	50 µL + 150 µL

The Cut-off indices are corrected by the dilution factors of each dilution in relation to the 1:101 dilution:
The Cut-off index for the 1:401 serum dilution must be multiplied by 4 and the 1:4 CSF dilution must be divided by 25.
A set of dilutions should be performed, if the test sample results are not within the range of 2.0 to 0.3 OD.
The following dilutions are recommended:

Serum	1:100	1:200	1:400	1:800	1:1600
CSF	1:2	1:4	1:8	1:16	1:32

⚠ **IgM samples must not be treated with RF-Absorbent, because the RF-Absorbent is already part of the Diluent Buffer. The time until the samples are dispensed should be < 15-20 min.**

TEST PROCEDURE

1. Pipette **100 µL** of **each Standard**, **Control and diluted sample** into the respective wells of the Microtiter Plate. In the qualitative test only Standard B (Cut-off Standard) is used.
2. **Incubate 1 h** at **37 °C**. Use cover or moisture chamber.
3. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Pipette 100 µL of Enzyme Conjugate into each well.
5. **Incubate 30 min** at **37 °C**. Use cover or moisture chamber.
6. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
7. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8. Pipette **100 µL** of **TMB Substrate Solution** into each well.
9. **Incubate 30 min at RT in the dark.**
10. Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
11. Measure optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws.
It is recommended to participate at appropriate quality assessment trials.
In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

CALCULATION OF RESULTS

The evaluation of the test can be performed either qualitatively or quantitatively.

C. Qualitative Evaluation

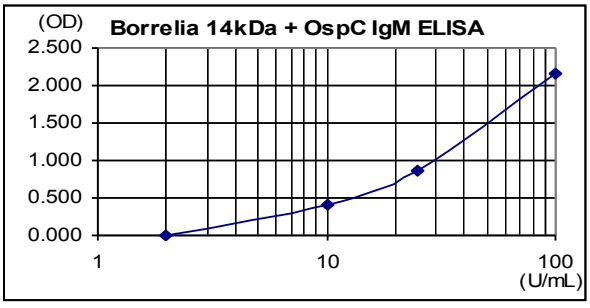
The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical density of the sample and Cut-off value. If the optical density of the sample is within a range of 10 % around the Cut-off value (grey zone), the sample has to be considered as borderline. Samples with higher ODs are positive; samples with lower ODs are low.
Typical Example:
Cut-off = OD (Standard B, Cut-off standard) = 0.45
Sample OD = 0.60
Cut-off index (COI): 0.60 / 0.45 = 1.33. The sample has to be considered positive.

D. Quantitative Evaluation

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.
For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).
The concentration of the samples can be read from the standard curve.
The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.
Samples showing concentrations above the highest standard can be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve
(Example. Do not use for calculation!)

Standard	U/mL	OD Mean
A	2	0.011
B	10	0.414
C	25	0.856
D	100	2.167



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(in Englisch via Internet unter DGHM.org oder NRZ-Borrelien.LMU.de).

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