Instructions for Use

TBE virus (FSME) IgG ELISA

Enzyme immunoassay for determination of IgG-antibodies against TBE virus in human serum and plasma.

REF **RE57401**

Σ **96**

2-8°C

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for determination of IgG antibodies against TBE virus in human serum and plasma. Control of humoral immune status and confirmation of seroconversion after vaccination ('Vaccination Management'). Identification of overt or latent TBE infection, usually with an additional anti-TBE-IgM determination. Antibody check following TBE infection. Confirmation of TBE versus Borreliosis following tick bite. Differential diagnosis of other CNS disorders.

2. SUMMARY AND EXPLANATION

In Europe, FSME (Tick-borne Encephalitis referred to as TBE) and Lyme Disease (Borreliosis) are the most frequent tick-borne infections. Borreliosis is very widespread but TBE is confined to special endemic regions (in Southern Germany, Thuringia, Austria, Switzerland, Hungary, Sweden, Czech, Slovak Republics, Croatia, Slovenia as well as some regions of former Soviet Union, etc.).

Both infections are similar in their development, consisting of two or more phases. The viraemic phase of TBE has an incubation period of 3-14 days with influenza-like symptoms in the first phase (1-8 days). After a non-febrile interval of about one week the infection may enter into a second phase, characterized by neurological symptoms of varying intensity. This stage may last for many weeks.

At the beginning of the second sickness phase, usually anti-TBE-IgM antibodies are detectable. Antibody levels reach their peak after 2-6 weeks. It can take 10 months for antibodies to fall below the level of detection. Anti-TBE-IgG antibodies are detectable simultaneously or a few days after the appearance of IgM-antibodies. Infection means immunity which mostly lasts a lifetime. Vaccination will also prevent the disease. Regular serological checks establish whether boosters are required ('vaccination management').

TBE-specific antibodies in Cerebrospinal Fluid (CSF) may be caused by a dysfunction of the hemato-encephalic barrier before or during an immune response to TBE antigens or may be the result of a local immune response. The antibody level fluctuations in the CSF may differ from those prevalent in serum/plasma.

3. TEST PRINCIPLE

TBE IgG is a two-step ELISA. Test wells in the ELISA test strips are coated with inactivated TBE virus. Diluted serum or plasma samples are incubated in the test wells of the test strips (**sample incubation**). During the incubation period specific antibodies against the TBE virus are bound to the solid phase. Non-specific components are washed away. Conjugate reaction takes place during the second incubation phase **(conjugate incubation)**. The anti-human-IgG peroxidase conjugate acts as a marker for the bound anti-TBE-IgG antibodies. Unbound conjugate is removed by a second washing step. In the third incubation phase the **substrate reaction** takes place. The peroxidase is part of the conjugate and oxidizes the substrate tetramethylbenzidine (TMB) into a blue colored substance. To stop the reaction sulphuric acid is added and the color will change to yellow. The color intensity is directly proportional to the anti-TBE-IgG-antibody concentration. The optical density is measured at a wavelength of 450 nm using an ELISA reader. Using the standard curve anti-TBE-IgG antibodies can be quantitatively evaluated.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* 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 IBL 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 on the IBL-Homepage or upon request directly from IBL.

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. 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. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sun light. The unopened reagents are stable until the expiry date indicated. The microtiter strips are stable until the expiry date, if they are stored at 2-8 °C in the tightly closed bag. The storage and stability of prepared reagents is stated in the corresponding chapters.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma

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 hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

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

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Ready to use. Break apart strips. Coated with inactivated TBE virus.
1 x 0.6 mL	ENZCONJ CONC	**Enzyme Conjugate** Concentrate Blue colored. anti-human IgG conjugated to peroxidase.
1 x 5 x 0.35 mL	CAL 1-5	**CAL 1-5** Concentrate Contains: Human serum, stabilizers, preservatives. **Concentrations are lot-specific as indicated on the bottle labels.**
2 x 0.35 mL	Control LL Control HL	**Control LL+HL** Concentrate Positive Control Serum, LL, “Low Level”, HL, “High Level”. Contains: Human serum, stabilizers, preservatives. **Concentrations are lot-specific as indicated on the bottle labels.**
2 x 75 mL	DILBUF	**Diluent Buffer** Ready to use. Red colored. Contains: detergents, 0.005 % (w/v) Thimerosal, 0.01 M Tris/HCl; pH 7.4.
1 x 100 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (10x) Contains: phosphate buffer.
2 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB (tetramethylbenzidine).
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. Contains: 0.5 M H_2SO_4.
2 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 5; 25; 50; 100; 500 µL
2. Vortex mixer
3. Tubes for sample dilution
4. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
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

9. 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 (18-25 °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. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. 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.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate 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.
8. 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.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of concentrated components (Example for 32 wells)

Dilute / dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
80 µL	ENZCONJ CONC	8 mL	DILBUF	1:101	Mix carefully.	18-25 °C	1 hour
10 mL	WASHBUF CONC	90 mL	bidist. water	1:10	Mix carefully.	2-8 °C	2 months

10.1. Dilution of Standards, Controls and Samples

	to be diluted	with	Relation	Remarks
CAL 1-5 Control LL Control HL	generally	DILBUF	1:101	e.g. 10 µL CAL/Control + 1000 µL
Serum, Plasma	generally	DILBUF	1:101	e.g. 10 µL Sample + 1000 µL
CSF	generally	DILBUF	1:9	e.g. 50 µL Sample + 400 µL

For the determination by standard curve calibrators 1-5 and control sera are needed. Samples containing concentrations higher than the highest standard have to be diluted further.

11. TEST PROCEDURE

1.	Pipette **200 µL** of diluted **Calibrator, Control and sample** into the respective wells of the Microtiter Plate.
2.	Cover plate with adhesive foil. **Incubate 1 h** at **RT (18-25 °C).**
3.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette **200 µL** of diluted **Enzyme Conjugate** into each well.
5.	Cover plate with adhesive foil. **Incubate 1 h** at **RT (18-25 °C).**
6.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 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 **200 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate 30 min** at **RT (18-25 °C).**
10.	Stop the substrate reaction by adding **50 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 ± 10 nm** (Reference-wavelength: 600-650 nm) within **10 min** after pipetting of the Stop Solution.

12. 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 comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. 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.

13. CALCULATION OF RESULTS

13.1. DETERMINATION OF STANDARD CURVE

Use enclosed (linear/linear) evaluation sheet

x-axis (log): Concentration in [VIEU*/mL]
y-axis (lin): Absorbance (optical density)

***VIE**NNA **U**NITS (Prof. Ch. Kunz/Vienna)

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 (e.g. 4 Parameter, equation 1).

Equation 1: $Y = d+(a-d)/(1+(x/c)^b)$

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. Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Validation criteria:
See QC-certificate.

13.2. INTERPRETATION OF SERUM-/ PLASMA SAMPLES

Samples with an absorbance exceeding that of the standard curve/calibrator 5 should be prediluted (1+1) with dilution buffer. The concentrations thus obtained have to be multiplied by the factor 2.
The conversion of citrate plasma to serum values is achieved by multiplying the recorded concentrations by the factor 1.1.

Assessment of anti-TBE-IgG antibodies:

< 63 VIEU/mL	negative
63-126 VIEU/mL	borderline
> 126 VIEU/mL	positive

14. INTERPRETATION OF RESULTS

14.1. Vaccination

Proven seroconversion (“immunization success” immunization management”) is determined by measurement of **anti-TBE-IgG antibodies** in sera or plasma.

1. Anti-TBE-IgG antibodies **negative**
   No latent immunization before vaccination.
   No seroconversion after vaccination. This may be the case after the first vaccination and, as an exception, also after the second and third vaccination or post-booster (no or low responders). If needed the basic immunization should be completed. Success or failure of vaccination should then be established by serological testing.
2. Anti-TBE-IgG antibodies **borderline level**
   This may be a case of seroconversion. Continue with basic immunization or booster. Repeat anti-TBE-IgG test within 2-4 weeks.
   This may be a non-specific reaction.
3. Anti-TBE-IgG antibodies **positive**
   This is a case of seroconversion.
   Check case history data of vaccination and, if needed, complete basic immunization or give a booster.

14.2. Infection

In order to confirm an infection, **anti-TBE-IgM antibodies** need to be determined. In order to ensure correct diagnosis **anti-TBE-IgG antibodies** should also be measured. If CSF samples are available, the presence of both anti-TBE-IgM and anti-TBE-IgG antibodies can be verified. When interpreting the serological results, the history of the patient has to be taken into account (stay in wooded area, tick bite, recent vaccination etc.).

1. Anti-TBE-IgM antibodies **and** anti-TBE-IgG antibodies **negative**
   In all likelihood there is no infection with the TBE virus. If such an infection is suspected repeat test within 7-10 days with new blood sample. A TBE infection may be either excluded or confirmed with a high degree of probability. A differential diagnosis for other infections of the CNS and, if there was a tick bite, Borreliosis should be considered.
2. Anti-TBE-IgM antibodies **negative** and anti-TBE-IgG antibodies **positive**
   There is either latent immunization or the infection occurred weeks or months before.
   If such an infection is suspected, a test for anti-TBE-IgM should be performed. A fresh TBE infection may be either excluded or confirmed with a high degree of probability.
3. Anti-TBE-IgM antibodies **positive** and anti-TBE-IgG antibodies **negative**
   A TBE virus infection is likely. After such an infection, IgM and subsequently IgG antibodies appear in the plasma. In the early phase of the infection the anti-TBE-IgG determination may at first be negative or reach borderline levels. A repeat test for anti-TBE-IgG (serum/plasma) within 7-10 days is recommended in order to detect any changes in antibody concentrations.
4. Anti-TBE-IgM antibodies **and** anti-TBE-IgG antibodies **positive**
   Most probably it is a case of TBE virus infection, provided there has not been any vaccination. The patient shows the typical symptoms of TBE but the intensity may vary.
   In case of borderline levels, blood has to be sampled again and the test repeated within 7-10 days. Any changes in antibody concentrations have to be monitored.

A serological diagnosis using CSF (CNS involvement) is only indicated if anti-TBE-IgM and anti-TBE-IgG antibody tests with serum/plasma are positive.

15. PERFORMANCE

Recovery of spiked serum samples: Variation of the theoretical expected value is ≤ 8 %.
For the intra-assay imprecision (n = 6-12) a coefficient of variation (CV) of < 8 % based on concentrations and < 7 % based on optical density was determined.
For the inter-assay imprecision (n = 5) a VK < 17 % was determined.

Specificity:
A panel of 235 „healthy“ patients with no fresh TBE Infection or immunization known in the case history was tested with one lot in duplicate measurements. Two patients were classified as false positive. The specificity as fraction of individuals without the disease who are tested negative is 99 %

Sensitivity:
A panel of 151 naturally infected patients was tested with one lot in duplicate measurements. One patient was tested false-negative. The sensitivity as fraction of patients harboring the disease who are tested positive is 94 %

Establishing cut-off levels:
Using a sample panel of different randomly selected samples (negative n = 91, immunized n = 68, infected n = 107) in stratified analysis empirical cut-off values (Prof. Ch. Kunz/Vienna) for anti-TBE-IgG anti-bodies were compared with those based on the balanced Youden index [8]. By applying this procedure, the empirical values of 63 VIEU/mL as the lower limit and 126 VIEU/mL as the upper limit of a grey zone were confirmed.
The sensitivity or specificity of the test outside of the borders of the grey zone was 97 or 99 % [8].

Interferences:
Haemolytic and lipaemic samples do not interfere with the test. Cross reactions of antibodies against other *flaviviridae* (e. g. Dengue Virus, Yellow fever Virus, West Nil Virus) may occur.

16. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

Symbol	Meaning
REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
i	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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