Instructions for Use

Tick-borne Encephalitis (TBE/FSME) Virus IgG ELISA

Enzyme immunoassay for the detection and quantitative determination of human IgG antibodies against Tick-borne encephalitis (TBE/FSME) virus in serum and plasma

1 x 96 well plate

ELS61239

Read the documentation

For Research Use Only

CONTENTS

1. INTRODUCTION

Tick-borne encephalitis (TBE) virus is a flavivirus of the family Togaviridae. It is an enveloped single-stranded RNA virus with cubic icosahedral symmetry and ranges in size from 20-80nm in diameter.

Three subtypes can be distinguished which show only little differences in their structural proteins.

TBE virus is mainly transmitted by ticks. The degree of contamination of ticks (and thus humans) in central Europe increases from west to east, and anybody may be affected. Specific antibody development yields a life-long immunity.

TBE is the most important tick-transmitted disease of man -beside Lyme disease, which is caused by the spirochete Borrelia burgdorferi. The clinical course of the disease depends on the immune status of the infected persons. A high virus production in the primary infected tissues is required for the passage of the blood-brain barrier and the resulting severe manifestations in the central nervous system.

Species	Disease	Symptoms (e.g.)	Transmission route
TBE/FSME Virus	Tick-borne encephalitis CEE (Central European Encephalitis)	Phase 1: unspecific flu-like symptoms (mild fever, headache, muscle pain, joint pain, gastrointestinal complaints) Phase 2: high fever, development of meningitis and / or encephalitis	By tick bites (Ixodes ricinus, western subtype; Ixodes persulcatus, eastern subtype). Rarely by infected (non-pasteurized) milk.

The presence of pathogen or infection may be identified by:

- Serology: e.g. ELISA, Neutralization, Hemagglutination Inhibition, Complement Fixation

2. INTENDED USE

The Tick-borne Encephalitis (TBE/FSME) Virus IgG ELISA is intended for the quantitative determination of IgG class antibodies against TBE virus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **TBE/FSME Coated Microplate (IgG):** 12 break apart 8-well snap-off strips coated with TBE/FSME Virus antigens; in resealable aluminium foil.
- **IgG Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 2 bottles containing 25 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **TBE/FSME anti-IgG Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5% NMP***. Note: NMP may damage an unborn child. Wear protective gloves and eye protection when handling TMB Substrate Solution. Get medical advice in case of exposure.***
- **TBE/FSME IgG Standards:** 5 vials, each containing 2 ml standard (human serum or plasma); coloured yellow; ready to use.

Standard A:	0	U/ml; blue cap	
Standard B:	50	U/ml; green cap	
Standard C:	130	U/ml; yellow cap	[U = Units]
Standard D:	200	U/ml; red cap	
Standard E:	300	U/ml; white cap	

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied
- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE
Store the kit at 2...8 °C. The opened 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 and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Coated Microplate
The break-apart snap-off strips are coated with TBE/FSME Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)
Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution
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.

7. SAMPLE COLLECTION AND PREPARATION
Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °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 10 µl sample and 1 ml 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 instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the 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 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is important! Insufficient washing results in poor precision and false results.

5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20…25°C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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 standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

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

9. RESULTS

9.1. Run Validation Criteria

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

- **Substrate blank: Absorbance value < 0.100**
- **Standard A: Absorbance value < 0.200**
- **Standard B: Absorbance value > 0.050**
- **Standard C: Absorbance value > Standard B**
- **Standard D: Absorbance value > Standard C**
- **Standard E: Absorbance value > 1.000**

Standard A < Standard B < Standard C < Standard D < Standard E

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

9.2. Calculation of Results

In order to obtain quantitative results in U/ml plot the (mean) absorbance values of the 5 Standards A, B, C, D and E on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 50, 130, 200 and 300 U/ml) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).

Read results from this standard curve employing the (mean) absorbance values of each sample.

For the calculation of the standard-curve mathematical Point to Point function should be used.

9.3. Interpretation of Results

Normal value ranges for this ELISA should be established by each laboratory.

The following values should be considered as a guideline:

Positive	> 110 U/ml	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	55 –110 U/ml	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative.**
Negative	< 55 U/ml	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	1.411	3.51
#2	24	1.991	2.23
#3	24	1.913	8.93

Interassay	n	Mean (U/ml)	CV (%)
#1	12	26.55	9.18
#2	12	15.38	12.99
#3	12	253.81	10.48

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 100.0% (95% confidence interval: 93.84% - 100.0%).

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 100.0% (95% confidence interval: 96.31% - 100.0%).

10.4. Analytical Sensitivity

The analytical sensitivity (according to CLSI EP17-A) is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator. It is 1.78 U/ml.

10.5. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.6. Cross Reactivity

Cross reactivity with other flaviviruses cannot be excluded and should be taken into account for result interpretation.

10.7. Measurement range

The measurement range is 1.78 U/ml – 300 U/ml.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- 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 samples.
- Only for research use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.

- 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.
- 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 standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

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

Prod. No.: ELS61239 Tick-borne Encephalitis (TBE/FSME) Virus IgG ELISA

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ABBREVIATIONS

NMP	N-Methyl-2-pyrrolidone

SYMBOLS KEY

LOT	Lot Number
	Expiration Date
	Storage Temperature
REF	Catalogue Number
	Consult Instructions for Use
MTP	Microplate
CONJ	Conjugate
CAL	Standard or Calibrator A-E
DIL G	IgG Sample Diluent
SOLN STOP	Stop Solution
SUB TMB	TMB Substrate solution
WASH BUF 20x	Washing Buffer 20x concentrated
	Contains sufficient for “n” tests

SCHEME OF THE ASSAY

Tick-borne Encephalitis (TBE/FSME) Virus IgG ELISA

Test Preparation

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

Assay Procedure

	Substrate Blank (A1)	Standard A	Standard B	Standard C	Standard D	Standard E	Sample (diluted 1+100)
Standard A	-	100µl	-	-	-	-	-
Standard B	-	-	100µl	-	-	-	-
Standard C	-	-	-	100µl	-	-	-
Standard D	-	-	-	-	100µl	-	-
Standard E	-	-	-	-	-	100 µl	-
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 Buffer							
Conjugate	-	100µl	100µl	100µl	100µl	100 µl	100µl
Incubate for 30 min at room temperature (20…25°C) Do not expose to direct sunlight Wash each well three times with 300µl of Washing Buffer							
TMB Substrate solution	100µl	100µl	100µl	100µl	100µl	100 µl	100µl
Incubate for exactly 15 min at room temperature (20…25°C) in the dark							
Stop Solution	100µl	100µl	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)							