The NativeAntigen COMPANY

Instructions for Use

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

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

1 x 96-well plate

ELS61240

Read the documentation

For Research Use Only

CONTENTS

1. INTENDED USE

The Tick-borne encephalitis (TBE/FSME) virus IgM antibody ELISA kit has been designed for the detection and the quantitative determination of specific IgM antibodies against Tick-borne encephalitis virus in human serum or plasma (citrate, heparin).

2. GENERAL INFORMATION

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 (Ixodes ricinus, western subtype; Ixodes persulcatus, eastern subtype). 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.

3. PRINCIPLE OF THE ASSAY

The qualitative 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. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

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 the responsibility of the user

- Only for in-vitro diagnostic 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.
- Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

5. REAGENTS AND MATERIALS PROVIDED

Reagent	Component	Volume / Qty
MTP	Antigen coated 8-well snap-off strips	12 x 8-well strips
CONJ	Enzyme Conjugate	1 x 20 mL
CON-	Negative Control	1 x 2 mL
CON+	Positive Control	1 x 2 mL
CUT OFF	Cut-off Control	1 x 3 mL
DIL G	Sample Diluent	1 x 80 mL
SOLN STOP	Stop Solution	1 x 15 mL
SUB TMB	TMB Substrate solution	1 x 15 mL
WASH BUF 20x	Washing Buffer 20x concentrated	2 x 25 mL
SUB TMB	TMB Substrate solution	1 x 15 mL

Storage and Stability

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 (20-25°C) and mixed thoroughly. After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened reagents are stable up to the expiry date stated on the label when stored at 2-8°C.

TBE/FSME Coated Microplate (IgM): 12 break-apart 8-well snap-off strips coated with TBE/FSME Virus antigens; in resealable aluminium foil.

IgM Sample Diluent: 1 bottle containing 80 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; 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.): 1 bottle containing 50 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-IgM Conjugate: 1 bottle containing 20 ml of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; 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 (N-Methyl-2-pyrrolidone). *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 IgM Positive Control: 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.

TBE/FSME IgM Cut-off Control: 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.

TBE/FSME IgM Negative Control: 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

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

6. MATERIALS REQUIRED BUT NOT PROVIDED

- 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

7. SAMPLES TO BE TESTED

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 at -20 °C or -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 IgM Sample Diluent. Dispense 10 µl sample and 1 ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and mix thoroughly with a vortex.

8. ASSAY PROCEDURE

8.1. Preparation of Reagents

It is very important to bring all reagents and samples to room temperature (20-25 °C) and mix them thoroughly before starting.

Coated Microplate: The break-apart snap-off strips are coated with TBE/FSME Virus antigen. 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.

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.

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.

8.2. Assay Steps
Please read the instructions for use carefully before performing the assay. Reliable results depend on strict adherence to the instructions for use, as described. The following test procedure is only validated for manual procedures. If performing the test on automatic ELISA 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.

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.

Assay Procedure

- 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.

Measurement

12. Adjust the ELISA microwell plate reader to zero using the Substrate Blank. If for 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.
13. Measure the absorbance of all wells at 450 nm and record the absorbance value for each standard/control and sample in the plate layout.
14. Bichromatic measurement using a reference wavelength of 620 nm is recommended.
15. Where applicable calculate the mean absorbance values of all duplicates.

9. EVALUATION

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

- Substrate Blank: Absorbance value < 0.100
- Negative Control: Absorbance value < 0.200 and < Cut-off
- Cut-off Control: Absorbance value 0.150 – 1.300
- Positive Control: Absorbance value > Cut-off

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

Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.
Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43. Cut-off = 0.43

Results in Units [U]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[arbitrary units = U]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ U (units)

Interpretation of Results

Cut-Off	10 U	Results
Positive	> 11 U	Antibodies against the pathogen are present. There has been contact with the antigen.
Equivocal	9 – 11 U	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	< 9 U	The sample contains no antibodies against the pathogen. Exposure to the antigen is unlikely.

10. ASSAY CHARACTERSTICS

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

Intra-assay	n	Mean (E)	CV (%)
#1	24	0.490	8.77
#2	24	1.039	7.77
#3	24	0.917	11.74
Inter-assay	**n**	**Mean (U)**	**CV (%)**
#1	12	18.14	10.86
#2	12	15.99	13.26
#3	12	2.04	12.56

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 **98.29%** (95% confidence interval: 95.07% - 99.65%).

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: 69.15% - 100.0%).

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.

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

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