FSME (TBEV) ELISA Kit

Enzyme Immunoassay for the determination of FSME (TBEV) in animal serum.

Catalog number: ARG83078

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

The disease is most often biphasic. After an incubation period of approximately one week (range: 4–28 days) from exposure (tick bite) non-specific symptoms occurs. These symptoms are fever, malaise, headache, nausea, vomiting and myalgias that persist for about 5 days. Then, after approximately one week without symptoms, some of the infected develop neurological symptoms, i.e. meningitis, encephalitis or meningoencephalitis. Myelitis also occurs with or without encephalitis.Sequelae persists for a year or more in approximately one third of people who develop neurological disease. Most common long-term symptoms are headache, concentration difficulties, memory impairment and other symptoms of cognitive dysfunction.

PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. A specific FSME antigens has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any FSME antibodies present is bound by the immobilized antigens. After washing away any unbound substances, an HRP-conjugated antibody is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C
Standard A-E (0, 50, 130, 200, 300 U/ml)	5 X 2ml (Ready-to-use)	4°C
HRP-Streptavidin solution	20 ml (Ready-to-use)	4°C
Sample Diluent Buffer	100 ml (Ready-to-use)	4°C
20X Wash Buffer	50 ml	4°C
TMB Substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP Solution	15 ml (Ready-to-use)	4°C
Plate sealer	1 e.a.	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- It is very important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run.
- Unused strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8 °C.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved. Mix well before dilution.
- The TMB Color developing agent should be colorless (or could have a slight blue tinge) and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- All materials should be equilibrated to room temperature (RT; 20-25°C) before use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Perform all assay steps in the order given and without any delays.
- Change pipette tips between the addition of different reagent or samples.
- For further internal quality control each laboratory should additionally use known samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at -20 or -70°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. 10 ml of 20X Wash buffer +190 ml of distilled water). Mix thoroughly by Use a magnetic stirrer. The diluted 1X wash buffer is stable for 5 days at room temperature (20-25 °C).
- Patient sample: Dilute patient sample 1:101 with Sample Diluent buffer before assay, mix well. (e.g. 5 µl of serum + 500 µl of Sample Diluent buffer)

  Note: The controls are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **controls, diluted samples (1:101)** into wells. Leave one well empty for the Substrate Blank. Cover the wells and incubate for **1h at 37°C**.
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes** (for automatic systems wash the wells for a total 5 washes). Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Avoid overflows from the reaction wells. Keep the wash buffer in the wells for **> 5 sec** before removal. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

   Note: If performing the test on ELISA automatic systems we recommend increasing the washing steps from **three** up to **five** and the volume of Washing Buffer from **300 µL** to **350 µL** to avoid washing effects.
4. Add **100 µl** of **HRP-conjugated antibody** into each well (except for the Substrate Blank well). Cover wells and incubate for **30 minutes at RT**. (Do not expose to direct sunlight)
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of **TMB Substrate Reagent** to each well. Incubate for **15 minutes** at **room temperature** in dark. A blue color occurs due to an enzymatic reaction.

7. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450 nm (and reference filter 620 nm) immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Adjust the ELISA Microtiterplate reader to zero using the Substrate Blank.
3. Bichromatic measurement using a reference wavelength of 620 nm is recommended.
4. In order for an assay to be considered valid, the following criteria must be met:

Substrate A:	OD <0.200
Substrate B:	OD > Substrate A
Substrate C:	OD > Substrate B
Substrate D:	OD > Substrate C
Substrate E:	OD >1.000
Substrate Blank:	OD <0.100

   If these criteria are not met, the test is not valid and must be repeated.
5. 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.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.

6. Normal value ranges for this ELISA should be established by each laboratory based on its own sample populations in the geographical areas serviced.

   The following values should be considered as a guideline:

Positive	> 30 U / ml	Antibodies against the pathogen are present.
Equivocal	20 ~ 30 U / ml	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	< 20 U / ml	The sample contains no antibodies against the pathogen.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV values of intra-assay precision is 3.55% and inter-assay precision is 10.39%.

Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analytic.

It is 97.37% (95% confidence interval: 86.19%-99.93%).

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analytic.

It is 100% (95% confidence interval: 78.2%- 100%)

Cross Reactivity

Cross-reactions cannot be excluded.

Interferences

Interferences are not significant interference effect observed up to concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.