Quick Start Guide: TBE/FSME Virus IgG ELISA [ELS61239]

Enzyme immunoassay for the detection and quantitative determination of human IgG antibodies against TBE/FSME Virus in serum and plasma (research use only).

(A) 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.
- 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).

(B) Assay Steps

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank. Cover wells with the foil supplied in the kit.
2. Incubate for 1 hour ± 5 min at 37 ± 1°C.
3. 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.
4. 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. Incubate for 30 min at room temperature (20-25°C). Do not expose to direct sunlight.
6. Repeat step 3.
7. Dispense 100 µl TMB Substrate Solution into all wells. Incubate for exactly 15 min at room temperature (20-25°C) in the dark. A blue colour occurs due to an enzymatic reaction.
8. 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.
9. Measure the absorbance of all wells at 450 nm within 30 min after addition of the Stop Solution and record the absorbance value for each standard/control and sample in the plate layout. Bichromatic measurement using a reference wavelength of 620 nm is recommended.