Tick borne encephalitis virus IgG ELISA Kit

**Cat. No.:** DEIA4304  
**Pkg.Size:** 96T

### Intended use

Tick borne encephalitis virus IgG ELISA assay is intended for research use of TBEV-associated diseases (encephalitis, meningoencephalitis). (Also, it can be used for differential test of neuroinfections and for monitoring of the antibody response after vaccination against TBEV. It is complementary examination to the detection of IgM anti TBEV (Tick borne encephalitis virus IgM ELISA Kit) and to the determination of IgG anti-TBEV avidity (Tick borne encephalitis virus IgG ELISA Kit).

### General Description

Tick borne encephalitis virus IgG ELISA Kit is detection IgG antibodies to tick-borne encephalitis virus in serum.

### Principle Of The Test

Tick borne encephalitis virus IgG ELISA Kit is a solid-phase immunoanalytical test. The polystyrene strips are coated with specific antigens which bear immunodominant epitopes of TBEV. Anti-TBEV antibodies from serum samples bind to the immobilized antigens. The other serum antibodies do not bind and are washed away, those that formed complexes with the antigens are later on recognised by animal anti-human IgG antibodies labelled with horseradish peroxidase. The presence of the labeled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background.

### Reagents And Materials Provided

- ELISA break-away strips coated with specific antigens: 96 wells
- 1.3 mL Positive control serum\(^1\), r.t.u.: 1 vial
- 1.3 mL Negative control serum, r.t.u.: 1 vial
- 0.2 mL Anti-human IgG antibodies labelled with horseradish peroxidase
  - 10x concentrated (Px-conjugate): 1 vial
  - 125 mL Wash buffer concentrate, 10x concentrated: 1 vial
- 125 mL Dilution buffer (DB), r.t.u.: 1 vial
- 15 mL Chromogenic substrate (TMB substrate), r.t.u.: 1 vial
- 15 mL Stop solution, r.t.u.: 1 vial
- Sealable pouch for unused strips
- Instruction manual
- Certificate of quality

\(^1\) ready to use

### Materials Required But Not Supplied

1. Distilled or deionised water for diluting of the Wash buffer concentrate.
2. Appropriate equipment for pipetting, liquid dispensing and washing.
Storage

1. Store the kit and the kit reagents at 2 to 10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.
2. Store undiluted serum samples at 2 to 10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.
3. Do not store diluted samples and a diluted Px-conjugate. Always prepare fresh.
4. Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.
5. Expiration date is indicated at the ELISA kit label and at all reagent labels.

Reagent Preparation

a. Allow all kit components to reach room temperature.
b. Vortex samples (sera) and Control sera in order to ensure homogeneity and mix all solutions well prior use.
c. Dilute serum samples 101x in Dilution buffer and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). Do not dilute Positive and Negative control serum, they are ready to use.
d. Prepare Wash buffer by diluting the Wash buffer concentrate 10x with an appropriate volume of distilled or deionised water (e.g. 100 mL of the concentrated Wash buffer + 900 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 - 37°C in a water bath. Diluted Wash buffer is stable for one week if stored at 2 to 10°C.
e. Dilute the concentrated Px-conjugate 101x with Dilution buffer (e.g. 0.1 mL Px-conjugate + 10 mL Dilution buffer). (Note: For one microtitre plate you will need approx. 12 mL of the diluted Px-conjugate, prepared by mixing 0,12 mL of the concentrated Px-conjugate + 12 mL of Dilution buffer).
f. Do not dilute TMB substrate and Stop solution, they are ready to use.

Assay Steps

a. Allow the microwell strips sealed inside the aluminium bag to reach room temperature to avoid moistening of the strips. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
b. Pipette Controls and samples according to the pipetting scheme (fig 1). Start with filling the first well with 100 µl of Dilution buffer (DB) to estimate the reaction background. Fill the next two wells with 100 µl of Positive control serum (PCS) and another well with 100 µl of Negative control serum (NCS). Fill the remaining wells with 100 µl of serum samples (S1, S2, S3, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply NCS and the samples in doublets and PCS in triplet.
c. Incubate 30 minutes (±5 min) at room temperature.
d. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells! If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.
e. Add 100 µL of diluted Px-conjugate into each well. Incubate 60 minutes (±5 min) at room temperature.
f. Aspirate and wash four times with 250 µl/well of Wash buffer (see point c of this paragraph)
g. Dispense 100 µl of TMB substrate into each well. Incubate 10 minutes (+/-5 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Keep the strips in the dark during the incubation with TMB substrate.
h. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents and to avoid bubbles.
i. Measure the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 630 nm.

**Evaluation**

Begin the processing with subtraction of the absorbance of the DB well (background absorbance) from the absorbances in all other wells.

A. Processing of results for the Qualitative interpretation

1. Compute the absorbance mean of the wells with Positive control serum (PCS). (If the PCS was applied in three parallels and in one the absorbance is different from the mean in more than 20% then exclude the deviating well from the calculation and compute a new absorbance mean with using the other two wells)

2. Compute the cut-off value by multiplying the mean of PCS with a Correction factor. Correction factor for this Lot is stated in Quality control certificate.

3. Serum samples with absorbances lower than 90% of the cut-off value are considered negative and samples with absorbances higher than 110% of the cut-off value are considered positive. The samples with absorbance in the range of 90-110% of cut-off value are equivocal.

B. Processing of results for the Semiquantitative interpretation

Determine Positivity Index for each serum sample as follows:

- Compute the cut-off value (see the previous paragraph)
- Compute the Positivity Index according to the following formula:
  \[
  \text{sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}
  \]

Express the serum reactivity according to Table 1 (Semiquantitative interpretation of results) Table 1: Semiquantitative interpretation of the results.

- negative: < 0.9
- +/-: 0.9 - 1.10
- +: >1.1

Note: An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.

**Reference Values**

The test is valid if:

- The background absorbance (the absorbance of the Dilution buffer) is less than 0.1
- The Positive control serum mean is higher than 0.8.
- The Negative control absorbance than 0.15

**Interpretation of Results**

When IgG(-) IgM(-) IgG Avidity(-), Seronegative, sensitive to the infection

When IgG(+) IgM(-) IgG Avidity(High), Anamnestic antibodies (past infection or the result of vaccination)

- Acute infection in vaccinated person*: second serum sample taken in 1-2 weeks after the first one should be tested

When IgG(-) IgM(+) IgG Avidity(-), Susp. early phase of acute infection: examination of the second serum sample taken in 1-2 weeks after the first one is recommended

When IgG(+) IgM(+) IgG Avidity(low), Acute primary infection

When IgG(+) IgM(-) IgG Avidity(low), Susp. acute or recent primary infection

When IgG(+) IgM(+) IgG Avidity(high), Susp. recent infection, infection in vaccinated individuals or unspecific
reactivity in IgM: examination of the second serum sample taken in two weeks and follow up of the antibodies dynamics is recommended

*Important note: Laboratory results can be interpreted only in the context with the patient’s symptoms and the clinical history.

Tick borne encephalitis virus IgG ELISA Kit may detect cross-reactive antibodies against the other flaviviruses, i.e., Dengue virus, West Nile, yellow fever or Japanese encephalitis viruses. Presence of IgG anti-TBEV antibody does not ensure protective immunity against TBEV infection. Presence of protective antibodies must be confirmed by virus-neutralisation test.

### Sensitivity

The diagnostic sensitivity was determined with the samples with expected positivity for IgG anti TBEV (vaccinated people, patients with acute or past TBEV infection). The diagnostic sensitivity of the test is 98.5%. Agreement with another commercial test was 98.5%.

### Specificity

The Diagnostic specificity was determined using TBEV-negative serum samples from unvaccinated blood donors. The specificity of the test was 100% and agreement with an alternative commercial test was 94.8%.

### Reproducibility

Intra-assay %CV: 2.5-4.0
Inter-assay %CV: 5.0-16.3

### Interferences

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 80 mg/mL of triglycerides.

### Precautions

**Handling Precautions**

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Follow the assay procedure indicated in the Instruction manual.
c. Controls, TMB substrate, Dilution buffer and Px-conjugate contain preservative Pr*Clin 300®.
d. Wash solution, TMB substrate, Stop solution and Dilution buffer are compatible and interchangeable between different ELISA sets except those with explicit statement in their Instruction manuals.
e. Avoid microbial contamination of serum samples and kit reagents.
f. Avoid cross-contamination of reagents.
g. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
h. Variations in the test results are usually due to:
   * Insufficient mixing of reagents and samples
   * Inaccurate pipetting and inadequate incubation times
   * Poor washing technique or spilling the rim of well with sample or Px-conjugate
   * Use of identical pipette tip for different solutions

### REFERENCES

1. Holzmann H: Diagnosis of tick-borne encephalitis. Vaccine 2003; S1: S1/36-S1/40.