Version 15-04/24

User's Manual

Tick borne encephalitis virus IgG ELISA Kit

REF **DEIA4304**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Tick borne encephalitis virus IgG ELISA Kit is for detection of IgG antibodies to tick-borne encephalitis virus in serum or cerebrospinal fluid.

Principles of Testing

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 recognized by animal anti-human IgG antibodies labeled 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

1. ELISA break-away strips coated with specific antigens. 96 wells
2. Standard A, negative control serum, ready to use. 1.3 mL
3. Standard D, calibrator, ready to use. 1.3 mL
4. Standard E, positive control serum, ready to use. 1.3 mL
5. Anti-human IgG antibodies labeled with horseradish peroxidase (Px-conjugate) ready to use. 13 mL
6. Wash buffer concentrate, 10× concentrated. 55 mL
7. Dilution buffer (DB), ready to use. 60 mL
8. Chromogenic substrate (TMB substrate), ready to use. 13 mL
9. Stop solution, ready to use. 13 mL

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.
3. Thermostat (set at 37°C) for ELISA plate incubation.
4. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

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. If you find damage at any part of the kit, please inform the manufacturer immediately.
4. Expiration date is indicated at the ELISA kit label and at all reagent labels.
5. The ELISA kit should be used within three months after opening.

Specimen Collection And Preparation

1. Vortex samples (sera, cerebrospinal fluids), Standards and controls in order to ensure homogeneity and mix all solutions well prior use.
2. Dilute serum samples 101× in Dilution buffer and mix well (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). Dilute cerebrospinal fluid samples 1:1 in Dilution buffer (e.g. 75 µL of the cerebrospinal fluid sample + 75 µL of the Dilution buffer). Do not dilute Control (Standards), they are ready to use.
3. Do not store diluted serum samples. Always prepare fresh.

Reagent Preparation

1. Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.
2. Prepare Wash buffer by diluting the Wash buffer concentrate 10× (WASH, 10×) with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 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 month if stored at room temperature.
3. Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.

Assay Procedure

Manufacturer will not be held responsible for results if manual is not followed exactly.

1. 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.
2. Pipette 100 µL of Dilution buffer, Standards, Control and serum samples to the wells according to the Plate Layout: Fill first well with Dilution buffer to determine reaction background. Fill the next two wells with Standard D. Fill the next well with positive control serum and negative control serum. The remaining wells fill with diluted tested sera (S1, S2, S3, S...) or cerebrospinal fluid samples (CSF1, CSF2, CSF3, CSF…). It is satisfactory to apply samples as singlets, however, if you want to minimize a laboratory error, apply control sera and tested sera as doublets, Standard D as triplet. Incubate 30 minutes (±2 min) at 37°C.
3. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells 4× 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.
4. Add 100 µL of Px-conjugate (ready to use) into each well. Incubate 30 minutes (±2 min) at 37°C.
5. Aspirate and wash 4× with 250 µL/well of Wash buffer. Aspirate and tap.
6. Dispense 100 µL of TMB substrate into each well. Incubate 15 minutes (±30 seconds) in dark 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.

7. 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.
8. Measure the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 620-690 nm.

Calculation

Processing of Results

Begin the processing of results with subtraction of the background absorbance (absorbance of the Dilution buffer well) from the absorbances of all other wells.

Processing of results for the Qualitative interpretation

1. Compute the absorbance (OD) mean of the wells with Standard D. If you applied Standard D into 3 wells and if any of the three Standard D absorbances falls out of the range +/-20% of the mean absorbance then exclude the deviating well from the calculation and compute a new Standard 1 mean using the values from the other two wells.
2. Compute the cut-off value by multiplying the OD mean of Standard D with Correction factor. Correction factor value for the particular Lot is written in Quality control certificate. The correction factor is different for serum samples and cerebrospinal fluid samples.

   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 (see note in Processing of results for the Semiquantitative interpretation).

Processing of results for the Semiquantitative interpretation

Determine Positivity Index for each serum samples and cerebrospinal fluid samples as follows:

1. Compute the cut-off value (see the previous paragraph)
2. Compute the Positivity Index according to the following formula:

   Sample Positivity Index = sample absorbance / cut - off value

3. Express the serum reactivity according to Semiquantitative interpretation of the results.

Serum samples and Cerebrospinal fluid samples

Positive Index	Interpretation
< 0.90	Negative
0.90 - 1.10	+/-
>1.10	Positive*

*on the basis of the Positive Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.

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 individual, usually withdrawn 1-2 weeks later.

Example of calculation:

Absorbance of Standard D =0.849; 0.895

Mean absorbance of Standard D =0.872

Correction factor for Standard D =0.26

Cut-off value =0.872 x 0.26=0.227

Absorbance of Serum sample =0.800

Positivity index =0.800/0.227=3.52

Interpretation Of Results

Presence of anti-TBEV antibodies			Interpretation
IgG	IgM	IgG Avidity	
-	-	-	Seronegative, sensitive to the infection
+	-	High	Anamnestic antibodies (past infection or the result of vaccination) Acute infection in vaccinated person*: second serum sample collected in 1-2 weeks after the first one should be tested
-	+	-	Suspect early phase of acute infection: examination of the second serum sample taken in 1-2 weeks after the first one is recommended
+	+	low	Acute primary infection
+	-	low	Suspect acute or recent primary infection
+	+	high	Suspect recent infection, infection in vaccinated individuals or unspecific reactivity in IgM: examination of the second serum sample collected in two weeks and follow up of the antibody dynamics is recommended

*Important note: Tick borne encephalitis virus IgG ELISA Kit may detect cross-reactive antibodies to 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-neutralization test.

Performance Characteristics

Validity of the test

The test is valid if:

The background absorbance (the absorbance of the Dilution buffer) is less than 0.150.

The mean absorbance values of standards/ control sera and the ratio between the absorbance values of Standard E/ Standard D are in the ranges stated in the Quality control certificate for this kit lot.

Precision

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.

Intraassay variability

The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

Example: (n = number of parallels of the same microtitration plate, SD = standard deviation)

n	Mean absorbance	SD	CV%
16	1.335	0.050	3.8%
16	0.614	0.023	3.7%

Interassay variability

The coefficient of intraassay variability is max. 15%. It is measured for each particular Lot as comparision of the OD values of the same serum sample in several consecutive tests.

Example: (n = number of an independent examination of the same serum sample, SD = standard deviation):

n	Mean Absorbance	SD	Range (min.-max.)	CV%
18	1.369	0.064	1.223-1.476	4.7%
43	1.372	0.119	1.184-1.750	8.7%

Sensitivity

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

Specificity

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

Recovery

Measured values of recovery test for every Lot are between 80-120% of expected values.

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

1. All ingredients of the kit are intended for laboratory use only.
2. Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV.
3. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.
4. Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine.
5. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.
6. The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g.

Incidur, Incidin, chloramin, …) in concentrations recommended by producer.

7. Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
8. Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.
9. Follow the assay procedure indicated in the Instruction manual.
10. Standards (Controls), TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300® .
11. Avoid microbial contamination of serum samples and kit reagents.
12. Avoid cross-contamination of reagents.
13. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
14. 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