ELISA-VIDITEST
anti-TBEV IgG (CSF)

REF ODZ-192 | Σ 96 tests | 2°C 10°C 2°- 10 °C

Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative, Quantitative

Type samples: Serum/Plasma/Cerebrospinal fluid

Possibility of determination: Intrathecal synthesis

Processing: Manual

CE IVD

VIDIA

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

ELISA-VIDITEST anti-TBEV IgG (CSF)

ODZ-192

Instruction manual

PRODUCER: VIDIA spol. s r.o., Nad Safinou II 365, 252 50 Vestec, Czech Republic,
Tel.: +420 261 090 565, www.vidia.cz, E-mail: info@vidia.cz

1. TITLE

ELISA-VIDITEST anti-TBEV IgG (CSF) - ELISA kit for the detection of IgG antibodies to tick-borne encephalitis virus in serum (plasma), cerebrospinal fluid or for estimation of the intrathecal antibody production.

2. INTENDED USE

ELISA-VIDITEST anti-TBEV IgG (CSF) kit is intended for in vitro diagnosis of TBEV- associated diseases (encephalitis, meningoencephalitis). It can be also used for differential diagnosis of neuroinfections and for monitoring of the antibody response after vaccination against TBEV. It is a complementary examination to the detection of IgM anti-TBEV (ELISA-VIDITEST anti-TBEV IgM) and to the determination of IgG anti-TBEV avidity (ELISA-VIDITEST anti-TBEV IgG avidity).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-TBEV IgG (CSF) 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.

4. KIT COMPONENTS

ELISA break-away strips coated with specific antigens STRIPS Ag	1 microplate
1.3 mL Standard A=negative control serum, r.t.u.[1] STA/NC	1 vial
1.3 mL Standard D=calibrator, r.t.u. STD/CAL	1 vial
1.3 mL Standard E=positive control serum, r.t.u. STE/PC	1 vial
13 mL Anti-human IgG antibodies labeled with horseradish peroxidase (Px-conjugate) r.t.u. CONJ	1 vial
55 mL Wash buffer concentrate, 10x concentrated WASH 10x	1 vial
60 mL Dilution buffer (DB), r.t.u. DIL	1 vial
13 mL Chromogenic substrate (TMB substrate), r.t.u. TMB	1 vial
13 mL Stop solution, r.t.u. STOP	1 vial
Instruction manual	
Quality control certificate	

[1] r.t.u. ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Chromogenic substrate TMB is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB and not with other Chromogenic substrates TMB-O, TMB-BF.

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

a. Distilled or deionised water for diluting of the Wash buffer concentrate.

b. Appropriate equipment for pipetting, liquid dispensing and washing.

c. Thermostat (set at 37°C) for ELISA plate incubation.

d. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES

a. Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.

b. **Vortex samples (sera (plasma), cerebrospinal fluids), Standards and controls in order to ensure homogeneity** and mix all solutions well prior use.

c. **Dilute serum (plasma) samples 101x in Dilution buffer** (DIL) 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 cerebrospinal fluid sample + 75 µL of Dilution buffer). **Do not dilute** the Control (Standards), they are ready to use.

d. Prepare **Wash buffer** by diluting the Wash buffer concentrate 10x (WASH, 10x) 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.

e. **Do not dilute** Px-conjugate, TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE

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

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 100 µL of Dilution buffer, Standards, Control and serum samples to the wells according to the pipetting scheme in Figure 1: Fill first well with Dilution buffer DIL to determine reaction background. Fill the next two wells with Standard D STD/CAL. Fill the next well with positive control serum STE/PC and negative control serum STA/NC. 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**.

c. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells 4x 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.

d. Add 100 µL of Px-conjugate r.t.u. CONJ into each well.

Incubate **30 minutes** (±2 min) **at 37°C**.

e. Aspirate and wash 4x with 250 µL/well of Wash buffer. Aspirate and tap.

f. 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 dark during the incubation with TMB substrate.

g. **Stop the reaction** by adding 100 µL of Stop solution STOP. 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.

h. 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.

Fig.1. Pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL**	**CSF1**										
b	**STD/CAL**	**CSF2**										
c	**STD/CAL**	**CSF3**										
d	**STE/PC**	**CSF…**										
e	**STA/NC**											
f	**S1**											
g	**S2**											
h	**S…**											

8. PROCESSING OF THE RESULTS

Begin the processing of results with subtraction of the background absorbance (absorbance of the DIL well) from the absorbances of all other wells. If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

8.1 Processing of results for the Qualitative interpretation.

1. Compute the absorbance (OD) mean of the wells with Standard D ST D/CAL. 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 D mean using the values from the other two wells.
2. Compute the cut-off value by multiplying the OD mean of ST D/CAL 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 par. 8.2)

8.2 Processing of results for the Semiquantitative interpretation

Determine Positivity Index for each serum sample 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:

$$\text{Sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}$$

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

Table 1: Semiquantitative interpretation of the results:

Serum samples:

Positivity index	Interpretation
< 0.90	Negative
0.90 – 1.10	+/-
> 1.10	Positive*

Cerebrospibal fluid samples:

Positivity index	Interpretation
< 0.90	Negative
0.90 – 1.10	+/-
> 1.10	Positive*

* on the basis of the Positivity 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 patient, 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

8.3 Quantitative antibody determination in serum samples in arbitrary units (AU/mL or VIEU/mL)

Determination of the antibody concentration in the test samples (AU/mL or VIEU/mL) using the program E-CALCULATOR (www.vidia.cz).

In case of determination of the antibody amount in the units, use the E-CALCULATOR program, which calculates the AU/ml or VIEU/mL (arbitrary units, Vidia Ltd. r.o.) using a calibration curve. To the E-CALCULATOR add OD values of controls and tested samples. For the correct calculation from the calibration curve, it is necessary to specify parameter B / Bmax for Standard D and then parameters A1, A2, C, gray zone range and Cmin and Cmax parameters that are specific to each lot of the kit and are stated in **the Quality Control Certificate**.
The program evaluates the results automatically. **The evaluation in arbitrary units for sera and cerebrospinal fluid is stated in the Quality Control Certificate**.

Note 1: The evaluation +/- means the equivocal. In such case it is recommended repeating the assay. If the result of the sample is in the grey zone again, use an alternative diagnostic method or initiate taking another blood sample 1-2 weeks later.
Note 2: The quantification is accurate only within the linear part of the calibration curve. If the sample OD is outside the linear interval (OD 0.300-2.000), it is necessary to repeat the test with more diluted serum samples to obtain the precise quantification.

8.4.1. Calculation of the antibody index (AI)

8.4.1.1 Calculate the ratio of the total IgG concentration in cerebrospinal fluid to the total IgG concentration in serum ($Q_{total\ IgG}$) and the ratio of the cerebrospinal albumin level to the serum albumin level ($Q_{total\ alb}$).

$$Q_{total\ IgG} = \frac{\text{total IgG in CSF}}{\text{total IgG in blood serum}} \quad Q_{total\ alb} = \frac{\text{albumin in CSF}}{\text{albumin in serum}}$$

8.4.1.2 Calculate the limiting quotient $Q_{lim\ IgG}$ which is the amount of IgG found in cerebrospinal fluid that can originate from the systemic circulation (hyperbolic function according to Reiber et al. Clin Chem 37/7, 1153-1160 (1991)).

Compute Q_{lim} using the equation:

$$Q_{lim\ IgG} = 0.93 * \sqrt{(Q_{total\ alb})^2 + 6 * 10^{-6}} - 1.7 * 10^{-3}$$

8.4.1.3 Calculate the ratio of concentration of specific IgG antibodies in CSF to concentration of specific IgG in serum $Q_{spec\ IgG}$.

$$Q_{spec.IgG} = \frac{\text{spec.IgG CSF} * \text{sample dilution}}{\text{spec.IgG serum} * \text{sample dilution}}$$

Where spec. IgG CSF is the concentration of specific antibodies in AU/mL in cerebrospinal fluid and spec. IgG serum is the concentration of specific antibodies in AU/mL in serum.

Example of calculation:

spec. IgG CSF = 38 AU/mL, sample diluted 2 times in dilution buffer

spec. IgG serum = 10 AU/mL, sample diluted 101 times in dilution buffer

$$Q_{spec.IgG} = \frac{38 * 2}{10 * 101} = 75.2 * 10^{-3}$$

8.4.1.4 Calculation of antibody index AI

a) If $Q_{total\ IgG} < Q_{lim\ IgG,}$ then calculate AI using the formula:

$$AI = \frac{Q_{spec.IgG}}{Q_{total\,IgG}}$$

b) If $Q_{total\ IgG} > Q_{lim\ IgG}$ compute AI using the formula:

$$AI = \frac{Q_{spec.IgG}}{Q_{lim\ IgG}}$$

E-CALCULATOR software for the calculation of results is available on request (free).

Note: Suitable software applications (e.g. EPI info 6) can be used for calculation of the specific antibody index.

8.4.2 Result interpretation (according to Reiber)

AI value	Interpretation
< 1.3	negative, intrathecal synthesis not proven
1.3 - 1.5	equivocal
> 1.5	positive, intrathecal synthesis proven

Note: If the sample absorbance is out of the calibration curve (above the upper limit), repeat the test with different sample dilution.

Note: If both serum and cerebrospinal fluid samples give negative results, do not count antibody index AI (intrathecal synthesis of specific antibodies is not expected).

9. INTERPRETATION OF THE 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 persons*: 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:** Laboratory results can be interpreted only in the context with the patient´s symptoms and the clinical history. ELISA-VIDITEST anti-TBEV IgG 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.

10. CHARACTERISTICS OF THE TEST

10.1 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 ST E/PC / ST D/CAL are in the ranges stated in the Quality control certificate for this kit lot.

10.2 Precision of the test

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

10.2.1 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, ±σ = standard deviation)

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

10.2.2 Interassay variability

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison 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, ±σ = standard deviation):

n	Mean Absorbance	±σ	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%

10.2.3 Recovery test

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

10.3 Diagnostic sensitivity and specificity

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%

The Diagnostic 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%.

10.4 Interference

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.

10.5 Limit of detection

The limit of detection was calculated as the minimal concentration that was at the 95% confidence level different from the Blank. The limit of detection is 1 AU/mL.

11. SAFETY PRECAUTIONS

All ingredients of the kit are intended for laboratory use only.

Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

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.

Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin,...) in concentrations recommended by the producer.

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.

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.

12. HANDLING PRECAUTIONS

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Follow the assay procedure indicated in the Instruction manual.
c. Calibrator and control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3:1)).
d. Wash buffer, Stop solution and Dilution buffer are compatible and interchangeable between different ELISA-VIDITEST kits except those with explicite 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

13. STORAGE AND EXPIRATION

The ELISA kit should be used within three months after opening.

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.

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.

Do not store diluted serum samples. Always prepare fresh.

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.

Expiration date is indicated at the ELISA kit label and at all reagent labels.

14. USED SYMBOLS

 number of tests

CE Conformité Européenne – product meets the requirements of European legislation

IVD in vitro diagnostics

±σ standard deviation

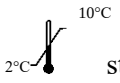
CV coefficient of variation

OD optical density

 manufacturer

 expiration

LOT Lot of kit

 storage at +2°C - +10°C

°C Celsius degree

% percentage

n number of tested samples

A valuea of tested sample

 read usage instructions

REF catalog number

References:

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

Dumpis U., Crook D., Oksi J.: Tick-Borne Encephalitis. Clinical Infectious Diseases, 1990; 28 (April): 882-890.

Heinz F. X., Roggendorf M., Hofmann H., Kunz Ch., Deinhardt F.: Comparsion of Two Different Enzyme Immunoassays for Detection of Immunoglobulin M Antibodies Against Tick-Borne Encephalitis Virus in Serum and Cerebrospinal Fluid. Journal of Clinical Microbiology, Aug. 1981; 141-146.

Niedrig M, Vaisviliene D, Teichmann A, et al. Comparison of six different commecial IgG-ELISA diagnostic kits for the detection of TBEV antibodies . J Clin Virol 2001;20:179-182.

15. FLOW CHART

Step 1.	**Prepare reagents and samples**
	↓
Step 2.	**Dispense 100 µL/well of Dilution buffer, Standards and samples**
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4x (250 µL/well), aspirate
	↓
Step 3.	**Dispense 100 µL/well of Px-conjugate r.t.u.**
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4x (250 µL/well), aspirate
	↓
Step 4.	**Dispense 100 µL/well of TMB substrate**
	↓
	Incubate 15 minutes in dark at room temperature
	↓
Step 5.	**Dispense 100 µL/well of Stop solution**
	↓
Step 6.	**Read the absorbance at 450/ 620-690 nm within 10 min**

The development of this kit was supported by grant from Ministry of Industry and Trade of the Czech Republic.

Date of the last revision of this manual: 02/2021