ELISA-VIDITEST anti-HSV 1+2 IgG (CSF)
(quantitative)

Cat. No.: ODZ-167

Instruction manual

**Producer:** VIDIA spol. s r.o., Nad Safinou II/ 365, Vestec, 252 42 Jesenice, Czech Republic, Tel.: +420 261 090 565, Web: www.vidia.cz, E-mail: info@vidia.cz

1. **TITLE:**
ELISA-VIDITEST anti-HSV 1+2 IgG (CSF) – ELISA kit for quantitative detection IgG antibodies to herpes simplex virus type 1 and 2 in human serum and cerebrospinal fluid (liquor).

2. **INTENDED USE:**
ELISA-VIDITEST anti HSV 1+2 IgG (CSF) is intended for in vitro diagnosis of HSV type 1 or 2 associated diseases, i.e. herpes labialis, herpes genitalis, herpesvirus gingivostomatitis, keratoconjunctivitis and herpesvirus-induced neurological complications (encephalitis, meningitis, inflammatory mono- and polyneuropathies). The diagnostic kit can be also utilized for differential diagnosis of neuroinfections, infections of eye and skin exanthematous diseases.
The test does not differentiate HSV-1 and HSV-2 types.
For additional serological testing we recommended determination of IgM and IgA anti-HSV antibodies (ELISA-VIDITEST anti-HSV1+2 IgM, ELISA-VIDITEST anti-HSV1+2 IgA).

3. **TEST PRINCIPLE:**
ELISA-VIDITEST anti HSV 1+2 IgG (CSF) is a solid-phase immunoanalytical test. The polystyrene strips are coated with a mixture of antigens which bear immunodominant epitopes of HSV1 and HSV2. Anti-HSV1+2 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.
Detection of intrathecal production of antibodies (detection of specific IgG antibodies in cerebrospinal fluid produced intrathecally) is necessary for the assessment of the antibody response within the central nervous system. It requires parallel measurements of specific IgG antibodies in serum and in cerebrospinal fluid sample taken from the patient at the same time and the determination of total albumin and total IgG in both samples. The calculation of the specific antibody production is according to Reiber equation (see 8.2 – processing of the results).

4. **KIT COMPONENTS:**
ELISA break-away strips coated with specific antigens **STRIPS Ag** 1 microplate
1.3 mL Standard A (St A), 0 AU 1\(^1\)/ ml, r.t.u. **STANDARD A** 1 vial
1.3 mL Standard B (St B), 15 AU/ ml, r.t.u. **STANDARD B** 1 vial
1.3 mL Standard C (St C), 125 AU/ ml, r.t.u. **STANDARD C** 1 vial
1.3 mL Standard D (St D), 375 AU/ ml, r.t.u. **STANDARD D** 1 vial
1.3 mL Standard E (St E), 800 AU/ ml, r.t.u. **STANDARD E** 1 vial
13 mL Anti-human IgG antibodies labeled with horseradish peroxidase r.t.u (Px-conjugate) CONJ 1 vial
125 mL Wash buffer concentrate, 10x concentrated WASH 10x 1 vial
100 mL Dilution buffer, r.t.u. DIL 1 vial
13 mL Chromogenic substrate (TMB substrate), r.t.u. TMB 1 vial
15 mL Stop solution, r.t.u. STOP 1 vial
Sealable pouch for unused strips
Instruction manual
Quality control certificate

1) AU, artificial units
2) r.t.u., ready to use

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

6. PREPARATION OF REAGENTS AND SAMPLES:
   a. Allow all kit components to reach room temperature.
   b. Vortex samples (sera and cerebrospinal fluids) and Standards 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). For evaluation of the intrathecal production test two dilutions of serum samples: 101x and 404x. Dilution 404x prepare by 4x diluting the 101x diluted serum sample (e.g. 150 μL of Dilution buffer + 50 μL of serum sample diluted 101x). Dilute cerebrospinal fluid samples 2x in Dilution buffer (e.g. 100 μL of cerebrospinal fluid sample + 100 μL of Dilution buffer). Do not dilute the Standards, 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 to +37°C in a water bath. Diluted Wash buffer is stable for one week if stored at +2 to +10°C.
   e. Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE:
   7.1. Procedure for the quantitative determination of antibodies in serum:
   a. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. 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 Standards and samples according to the pipetting scheme (page 4, fig 1). Start with filling the first well with 100 μl of Dilution buffer (DIL) to estimate the reaction background. Fill the next wells with 100 μl of Standard A, B, C, D and E. Fill the remaining wells with 100 μl of serum samples (S1, S2, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the samples in doublets. We recommend including a positive reference serum sample (in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.
   c. Incubate 60 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. Mix Px-conjugate r.t.u. well and then add 100 µL of Px-conjugate into each well.

f. **Incubate 60 minutes (±5 min) at room temperature.**

g. Aspirate and wash four times with 250 µL/well of Wash buffer.

h. Dispense 100 µl of TMB substrate into each well.

i. 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.

j. 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.

k. Measure the absorbance at **450 nm with a microplate reader within 10 minutes.** It is recommended to use a reference reading at 630 nm.

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7.2. Procedure for serum and cerebrospinal fluid samples (detection of intrathecal antibodies):

a. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. 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 Standards and samples according to the pipetting scheme (page 4, fig 2). Start with filling the first well with 100 µl of Dilution buffer (DIL) to estimate the reaction background. Fill the next wells with 100 µl of Standard A, B, C, D and E. Fill the remaining wells with 100 µl of serum samples (S1, S2, ...) and cerebrospinal fluid samples (CSF1, CSF2, ...). We recommend testing each serum sample in two different dilutions: 101x and 404x and each cerebrospinal fluid sample in one dilution: 2x. If you wish to minimize laboratory error apply the samples in doublets. We recommend including a positive reference serum sample (in-house internal control) into each run to follow the sequence, variability and accuracy of calibration. If many samples are tested, we recommend filling the samples into an auxiliary microplate and then transfer the samples to testing microplate using 8-channel pipette.

c. **Incubate 60 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. Mix Px-conjugate r.t.u. well and then add 100 µL of Px-conjugate into each well.

f. **Incubate 60 minutes (±5 min) at room temperature.**

g. Aspirate and wash four times with 250 µL/well of Wash buffer.

h. Dispense 100 µl of TMB substrate into each well.

i. Incubate for 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.

j. 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.

k. Measure the absorbance at **450 nm with a microplate reader within 10 minutes.** It is recommended to use a reference reading at 630 nm.
Figure 1: Pippetting scheme

Quantitative antibody determination in serum samples

<table>
<thead>
<tr>
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Figure 2: Pippetting scheme

Measurement of intrathecal antibody production (serum and CSF samples)

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<td>b</td>
<td>ST A</td>
<td>S2 (404x)</td>
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<td>c</td>
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<td>CSF1 (2x)</td>
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<td>d</td>
<td>ST C</td>
<td>CSF2 (2x)</td>
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<td>S2</td>
<td>(101x)</td>
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8. PROCESSING OF RESULTS:

Anti-HSV IgG antibodies determination in cerebrospinal fluid can be interpreted only using Intrathecal synthesis calculation.

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

8.1 Processing of results for the quantitative determination in serum samples

Compute the sample antibody titre in artificial units (AU/ml) as follows:

1. Construct the calibration curve by plotting the absorbance of Standards (y-axis – in linear scale) to antibody concentration in artificial units (AU/ml) (x-axis – may have logarithmic scale). The antibody concentration in each Standard (A-E) is mentioned in paragraph 4 – Kit components.

2. Determine the unknown antibody titre in the samples from the calibration curve. It is possible to use various softwares for the standard curve fitting and for the calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is the most convenient.
Result interpretation:

<table>
<thead>
<tr>
<th>Concentration (AU/mL)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 13</td>
<td>Negative</td>
</tr>
<tr>
<td>13 – 18</td>
<td>+/-</td>
</tr>
<tr>
<td>&gt; 18</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Note 1: The quantification is accurate only within the linear part of the calibration curve. If the sample OD is outside the linear interval it is necessary to repeat the test with more diluted serum samples to obtain the precise quantification.

Note 2: 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.

8.2 Processing of results for estimation of the intrathecal antibody production

1. Calculate the anti-HSV 1+2 IgG antibody concentrations (AU/mL) for all tested samples
   a. Construct the calibration curve by plotting the absorbance of Calibrators (y-axis – in linear scale) to antibody concentration in artificial units (AU/ml) (x-axis – may have logarithmic scale). The antibody concentration in each Standard (A-E) is mentioned in paragraph 4 – Kit components.
   b. Determine the unknown antibody titre in the samples from the calibration curve. It is possible to use various softwares for the standard curve fitting and for the calculation of the unknowns, e.g. Winliiana, KimQ. For better fitting, the polynomic (four-parameter) function is the most convenient.
   c. If you use another dilution for the test samples you have to correct the antibody concentration according to the formula:
      \[ C_s = \frac{C_{cal} \times \text{sample dilution}}{100} \]
      
      \( C_s \) = antibody concentration in the sample, \( C_{cal} \) = antibody concentration from the calibration curve

      Example: Sample dilution 2x, \( C_{cal} = 40 \); \( C_s = \frac{(40 \times 2)}{100} = 0.8 \text{ AU/ml} \)

2. Calculate the Specific antibody quotient as follows:
   \[ Q_{spec} = \frac{\text{concentration of IgG anti-HSV 1+2 (AU/mL) in cerebrospinal fluid}}{\text{concentration of IgG anti-HSV 1+2 (AU/mL) in serum}} \]

3. Compute the Total antibodies quotient as follows:
   \[ Q_{total} = \frac{\text{concentration of total IgG (mg/mL) in cerebrospinal fluid}}{\text{concentration of total IgG (mg/mL) in serum}} \]

VIDITAB software for the calculation of results is available on request (free).
4. Compute the Albumine quotient as follows:

\[ Q_{\text{alb}} = \frac{\text{concentration of albumin (mg/mL) in cerebrospinal fluid}}{\text{Concentration albumin (mg/mL) in serum}} \]

5. Calculate the Limite quotient \( Q_{\text{lim}} \) that shows status of the hematoencephalic barrier according to the Reiber’s equation\(^1\):

Compute \( Q_{\text{lim}} \) using the equation:

\[ Q_{\text{lim}} (\text{IgG}) = 0.93 \times \frac{1}{\sqrt{(Q_{\text{alb}})^2 + 6 \times 10^{-6}}} - 1.7 \times 10^{-3} \]

6. Compute the Antibody Index AI:

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

\[ AI = \frac{Q_{\text{spec}}}{Q_{\text{total}}} \]

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

\[ AI = \frac{Q_{\text{spec}}}{Q_{\text{lim}}} \]


9. INTERPRETATION OF RESULTS:

9.1. Determination of anti HSV1+2 antibodies in serum samples

Anti-HSV IgG antibodies persist whole life in patient serum after primary infection. Most of the antibodies cross react with both type of HSV virus. Significant increase of antibody titre can be a result of the infection reactivation, but due to the recurrent character of reactivations is not always demonstrable. Serological results can be interpreted only in context with other laboratory tests results and with patient symptoms.

9.2. Determination of the intrathecal antibody production

Result interpretation:

- AI < 1.5: intrathecal antibody production not found
- AI 1.5 – 2: suspect intrathecal antibody production
- AI > 2.0: intrathecal antibody production proven

10. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST:

10.1 Validity of the test

The test is valid if:

a. The background absorbance (DIL = background absorbance) is less than 0.100.

b. The Standard D mean is in range stated in Quality control certificate.

c. The Standard absorbance values keep the order that: ST A < ST B < ST C < ST D < ST E
10.2 Precision of the test

10.2.1 Intraassay and interassay variability

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

In anti-HSV IgG-positive samples intra-assay and inter-assay variability coefficients (CV) did not exceed 5% and 15% of mean absorbance values, respectively.

An example of intra-assay variability (n= number of parallels determinations in the same test):

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.453</td>
<td>0.013</td>
<td>2.8 %</td>
</tr>
<tr>
<td>16</td>
<td>1.079</td>
<td>0.030</td>
<td>2.7 %</td>
</tr>
</tbody>
</table>

An example of inter-assay variability (n= number of determinations in several independent tests):

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>SD</th>
<th>min – max</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.142</td>
<td>0.021</td>
<td>0.121 – 0.169</td>
<td>14.8%</td>
</tr>
<tr>
<td>10</td>
<td>0.534</td>
<td>0.046</td>
<td>0.474 – 0.626</td>
<td>8.6%</td>
</tr>
<tr>
<td>10</td>
<td>0.864</td>
<td>0.072</td>
<td>0.756 – 0.998</td>
<td>8.3%</td>
</tr>
<tr>
<td>10</td>
<td>2.089</td>
<td>0.115</td>
<td>1.990 – 2.334</td>
<td>5.5%</td>
</tr>
</tbody>
</table>

10.2.2 Spiking recovery test

The analytical recovery was measured by testing samples prepared as a mixture of a two samples with different absorbance values. The percentage of recovery was between 80-120% of theoretical value of the mixture.

10.3 Diagnostic sensitivity and specificity

The diagnostic sensitivity of the test is 98.8% and the specificity is 97%. Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

<table>
<thead>
<tr>
<th>HSV 1+2 status</th>
<th>Total</th>
<th>Positive</th>
<th>Equivocal</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>102</td>
<td>3</td>
<td>2</td>
<td>97</td>
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<tr>
<td>Positive</td>
<td>82</td>
<td>80</td>
<td>1</td>
<td>1</td>
</tr>
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</table>

* Equivocal results were not taken in account when calculating

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 50 mg/mL of triglycerides.

10.5. Linearity of the test

Calibration curve is linear within the OD range 0.3 – 2.000 (12 – 800 AU/ml)

10.4. Limit of quantification

The limit of quantification is 2 AU. The limit of quantification was defined as the lowest measurable amount of AU which can be distinguished with 95% confidence from zero.
11. SAFETY PRECAUTIONS:

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

Manufacturer guarantees performance of the entire ELISA kit. Follow the assay procedure indicated in the Instruction manual. Standards, TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®. Wash buffer, chromogenic substrate TMB, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.

Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces. 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:

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 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.
References:


14. FLOW CHART:

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Prepare reagents and samples</th>
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<tbody>
<tr>
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<tr>
<td>Step 2</td>
<td>Dispense 100 µL/well of Dilution buffer, Standards and samples</td>
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<td></td>
<td>Incubate 60 minutes at room temperature</td>
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<td>Wash 4 times (250 µL/well), aspirate</td>
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<tr>
<td>Step 3</td>
<td>Dispense 100 µL/well of Px-conjugate r.t.u.</td>
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<td>Incubate 60 minutes at room temperature</td>
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<td></td>
<td>Wash 4 times (250 µL/well), aspirate</td>
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<tr>
<td>Step 4</td>
<td>Dispense 100 µL/well of TMB substrate</td>
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<td>Incubate 10 minutes at room temperature in dark</td>
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<tr>
<td>Step 5</td>
<td>Dispense 100 µL/well of Stop solution</td>
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<tr>
<td>Step 6</td>
<td>Read the absorbance at 450 nm within 10 min.</td>
</tr>
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</table>

Date of the last revision of this manual: 07/2013