ELISA-VIDITEST human anti-β III TUBULIN IgM

ODZ-030

Instruction manual

PRODUCER:
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1. TITLE:
ELISA-VIDITEST human anti-β III TUBULIN IgM
Kit for detection of human IgM class antibodies against human tubulin βIII

2. INTENDED USE:
Kit is designed for semiquantitative detection of human IgM class antibodies against human tubulin β class III in human serum.
Kit is intended for research use only

3. TEST PRINCIPLE:
ELISA-VIDITEST human anti-β III TUBULIN IgM is an indirect solid phase enzyme-linked immunosorbent assay. Surface of microtitration wells is coated with antigen (C-terminal part of tubulin beta class III conjugated to a protein carrier). Anti-tubulin antibodies present in serum samples form complexes with the immobilised antigen. Anti-tubulin antibodies are detected with horseraddish peroxidase labelled anti-human animal antibodies. The amount of bound labelled antibodies is determined by colour reaction. Serum without anti-tubulin antibodies does not react and minor changes in colour represent background of the assay.

4. KIT COMPONENTS
12x 8-well colourless snap-off strips coated with antigen STRIPS Ag 1 microplate
6x 8-well colourless snap-off strips coated with control antigen (calibration strips) STRIPS CAL ½ microplate
1.3 mL Calibrator CAL 2 vials
1.3 mL Negative control CONTROL 1 r.t.u. 1 vial
1.3 mL Positive control CONTROL + r.t.u. 1 vial
0.2 mL 100x concentrated Anti-human Pxi conjugate labelled with horseraddish peroxidase CONJ 100x 1 vial
125 mL Wash buffer 10x concentrated WASH 10x 1 vial
125 mL serum IgM Dilution buffer r.t.u. DIL 1 vial
15 mL TMB substrate r.t.u. TMB 1 vial
30 mL Stop solution r.t.u. STOP 1 vial
Sealable pouch 1 piece
Instruction manual 1 r.t.u., ready-to-use

Dilution Buffer (DIL) is intended only for ELISA-VIDITEST human anti-β III TUBULIN IgM kit and IS ARE NOT COMPATIBLE with other ELISA-VIDITEST kits produced by VIDIA s r.o..
5. **Materials needed but not provided:**
Deionised water
Precision pipettes
Microplate washer or multichannel pipette
Microplate reader (wavelength 450 nm)
Plastic microplate cover or a sheet of an adhesive tape

6. **Preparation of reagents and serum samples:**
   a. Allow samples and components to reach room temperature prior to the assay.
   b. Agitate samples, controls and Calibrator in order to ensure homogeneity and mix the components well prior to use in the assay. Dilute serum samples with serum IgM Dilution buffer (DIL) 1:100 and mix (for example 5 μL of serum + 500 μL of serum IgM Dilution buffer). Do not dilute Controls and Calibrator, they are ready to use.
   c. Prepare enough Wash buffer by diluting 10x concentrated Wash buffer with deionised water (for example 100 ml 10x concentrated Wash buffer + 900 ml dH2O). If there are crystals of salt present in the Wash buffer concentrate, warm up the vial to 32-37°C in a water bath. Mix well before diluting. Diluted Wash buffer is stable for one week if stored at 2-10°C.
   d. Dilute concentrated Anti-human Px-conjugate 100x (CONJ 100x) with the serum IgM Dilution buffer (DIL) (for example 0.1 mL Px-conjugate + 10 mL of serum IgM Dilution buffer)
   
   **Note:**
   For 12x 8-well strips (one microtitre plate) you will need approx. 12 mL of diluted Px-conjugate.
   e. Do not dilute TMB substrate and Stop solution. They are ready to use.

7. **Assay procedure:**
   a. Allow the strips sealed in an aluminium bag to reach room temperature before opening it in order to prevent water condensation within the wells. Withdraw the adequate number of colourless strips for serum samples STRIPS Ag and one colourless strip STRIPS CAL for Calibration standard. If not all the strips are used, put the unused strips in the sealable pouch and keep the desiccant inside.
   b. Pipette 100 μL of serum IgM Dilution buffer DIL (blank), Calibrator, Controls and diluted samples per well according to the Table 1. It is satisfactory to use single well per sample, but duplicates are recommended to exclude laboratory error.
   c. Cover the wells and incubate for 60 minutes (+/- 5 minutes) at room temperature.
   d. Aspirate the contents of wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions) and wash four times (4x) with at least 250 μL per well of diluted Wash buffer.
   e. Pipette 100 μL of diluted Px-conjugate to each well.
   f. Cover the wells and incubate for 60 minutes (+/- 5 minutes) at room temperature.
   g. Aspirate and wash four times (4x) with 250 μL of diluted Wash buffer.
   h. Add 100 μL of TMB substrate into each well. Incubate for 10 minutes (+/- 5 seconds) at room temperature.
   i. Cover the strips with aluminium foil or else keep them in the dark during the enzymatic reaction.
   j. Add 100 μL of Stop solution. Tap gently the microplate few times to ensure complete mixing of the reagents.
   k. Determine the absorbance at 450 nm in a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.
8. **PROCESSING OF RESULTS:**

**Semiquantitative method**

1. Subtract the absorbance of DIL well A1 (blank) from the absorbance of Controls and subtract the absorbance of DIL well A2 from the absorbance of samples.
2. Calculate the mean absorbance of Calibrator (CAL).
   
   If a value of any CAL well differs from calculated mean in more than 20% of the mean, calculate a new mean value of CAL using the other two remaining wells.
3. Calculate the cut-off value for serum samples. The cut-off value is computed by multiplication of the CAL mean by a Correction factor. The Correction factor determines cut-off value according to the manufacturer’s testing during ELISA production. The Correction factor is a predefined cut-off/CAL ratio and is supplied for each lot of kits. The Correction factor adjusts the cut-off value to actual kit performance and eliminates day-to-day fluctuations due to changes in room temperature and inaccuracy in incubation times. **Correction factor value for the particular Lot is written in Quality control certificate.**
4. For each sample determine an Index of optical density as a ratio of sample absorbance and the calculated cut-off value. Use following formula:
   
   $$\text{Index} = \frac{\text{absorbance of sample}}{\text{cut-off}}$$
5. Classify serum reactivity according to Table 2.

Table 2

<table>
<thead>
<tr>
<th>Index value</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.00</td>
<td>Negative</td>
</tr>
<tr>
<td>1.00 – 1.20</td>
<td>+/-</td>
</tr>
<tr>
<td>&gt; 1.20</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Example:

Serum sample

- Absorbance of CAL: 1.040; 1.020; 1.065
- Mean absorbance of CAL: 1.042
- Correction factor: 0.26
- Cut-off value: 1.042 x 0.26 = 0.271
- Serum sample absorbance: 0.475
- Index value: 0.475 / 0.271 = 1.75
9. LIMITATIONS OF PROCEDURE, SPECIFICITY AND SENSITIVITY OF THE TEST

9.1. Validity of the test

The test is valid if:
- Absorbance of DIL (blank) is < 0.050.
- The mean absorbance of Calibrator (CAL) should be in range that is written in enclosed Quality control certificate.
- Mean absorbance of CONTROL + (blank already subtracted) is > 1.000
- The absorbance of CONTROL - (blank already subtracted) is less than or equal to 0.100.

9.2. Performance characteristics

Example of intraassay variability for different absorbance values (N = number of duplicates):

<table>
<thead>
<tr>
<th>N</th>
<th>ABS</th>
<th>Standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.215</td>
<td>0.009</td>
<td>4.4</td>
</tr>
<tr>
<td>16</td>
<td>0.384</td>
<td>0.011</td>
<td>2.8</td>
</tr>
<tr>
<td>16</td>
<td>0.556</td>
<td>0.019</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Example of interassay variability

<table>
<thead>
<tr>
<th>N</th>
<th>Mean Absorbance</th>
<th>Standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.065</td>
<td>0.007</td>
<td>11.3</td>
</tr>
<tr>
<td>10</td>
<td>1.103</td>
<td>0.069</td>
<td>6.3</td>
</tr>
<tr>
<td>10</td>
<td>1.659</td>
<td>0.072</td>
<td>4.3</td>
</tr>
</tbody>
</table>

10. SAFETY 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. However they should be regarded as contagious and handled and disposed of according to appropriate regulations. Autoclave all reusable materials that were in contact with human samples, incinerate disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine.
3. Neutralize liquid wastes containing Stop solution. Handle the Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with the Stop Solution wash skin thoroughly with water.
4. Do not pipette by mouth. Do not smoke, eat or drink in the area where samples or kit reagents are handled.
5. Wear disposable gloves while handling kit reagents or specimens and wash your hands thoroughly afterwards.
6. Avoid producing aerosol.

11. WARANTY AND PRECAUTIONS:

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Wash buffer, TMB substrate, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.
c. Avoid microbial contamination of serum samples and kit reagents.
d. Avoid cross-contamination of reagents during withdrawing, dilution and storage of reagents.
e. Avoid any contact of the TMB substrate with oxidizing agents or metal surfaces.
g. Follow the assay procedure indicated in the Instruction manual.

Variations in test results are usually due to:
- Insufficient mixing of reagents and samples prior use
- Inaccurate pipetting and inadequate incubation times
- Poor washing technique or spilling of samples or Px-conjugate at the rim of well
- Use of identical pipette tip for different solutions
12. REAGENTS STORAGE:

a. Store the ELISA kit and kit reagents at 2-10°C in a dry place and protected from light. Expiry date is indicated at the kit label and all reagent labels.

b. Store unused strips in the sealable pouch and keep the desiccant inside.

c. Store serum samples at 2-10°C up to one week. For longer periods make aliquots of undiluted serum/CSF and keep them at -20°C. Avoid repeated thawing and freezing.

d. Diluted serum samples and diluted Px-conjugate can not be stored. Always prepare fresh dilutions.

e. VIDIA ELISA kits are shipped in cooling bags. If you find damage at any part of the ELISA kit, please inform the manufacturer.

13. FLOW CHART

<table>
<thead>
<tr>
<th>Step 1. Dilute reagents and samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2. Add 100 µL/well of DIL, calibrator, controls and samples</td>
</tr>
<tr>
<td>Incubate 60 minutes at room temperature</td>
</tr>
<tr>
<td>Wash 4 x 250 µL/well</td>
</tr>
<tr>
<td>Step 3. Add 100 µL/well of Px-conjugate</td>
</tr>
<tr>
<td>Incubate 60 minutes at room temperature</td>
</tr>
<tr>
<td>Wash 4 x 250 µL/well</td>
</tr>
<tr>
<td>Step 4. Add 100 µL/well of TMB substrate</td>
</tr>
<tr>
<td>Incubate 10 minutes at room temperature</td>
</tr>
<tr>
<td>Step 5. Add 100 µL/well of Stop solution</td>
</tr>
<tr>
<td>Step 6. Read absorbance at 450 nm within 20 minutes</td>
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</tbody>
</table>

14. References:

Date of the last revision: 04/2013

For research purposes only