Varicella-Zoster Virus IgG ELISA Kit
Catalog No: E-HD-E008
96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
**Test principle**

This ELISA kit adopts Indirect-ELISA method as its principle. The micro ELISA plate provided in this kit has been pre-coated with inactivated purified varicella-zoster virus antigen. When samples are added into the micro ELISA plate wells, the anti-varicella-zoster virus IgG antibody in the sample will combine with the pre-coated mixed antigen to form antigen-antibody compound. Free components will be washed away. HRP conjugated Mouse-anti -human IgG monoclonal antibody is added to each well and react with the compound to form “antigen-antibody-HRP conjugated antibody” compound. Free components will be washed away. The TMB substrate solution is added to initiate the color developing reaction. The shade of developed color is proportional to the concentration of varicella-zoster virus IgG antibody.

**Experimental instrument**

Micro-plate Reader with 450 nm wavelength filter
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ incubator or water bath
Deionized or distilled water
Absorbent paper

**Kit components**

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Micro-plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>10 mL</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>50 mL</td>
</tr>
<tr>
<td>20×Concentrated Wash Buffer</td>
<td>30 mL</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>5.0 mL</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>5.0 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>5.0 mL</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1</td>
</tr>
</tbody>
</table>
Requirements of sample

1. Human serum/plasma or whole blood can be used as sample. Anticoagulants (heparin, EDTA, sodium citrate) has no interference to the test results.

2. Serum/plasma samples can be kept stable for 4 days at 2~8°C, but it is recommended to be used within 72 hours. Whole blood sample should be used immediately after collecting. Serum/plasma samples can be kept stable for 1 year when stored at -20°C immediately for long-term storage, while whole blood samples should be diluted with Sample Diluent and store the supernatant at -20°C for 1 year. It is suggested that the freeze-thaw cycles should be no more than 3 times.

3. Avoid using samples with hemolysis or lipidemia. Polluted samples can't be detected.

Assay procedure

1. Sample preparation:
   (1) Add 500 μL of Sample Diluent into 1.5 mL centrifuge tube. Label the tubes.
   (2) Collect 10 μL of whole blood, or take 5 μL of serum/plasma. Then add the samples into corresponding centrifuge tube, gently shake the tubes to mix thoroughly.
   (3) For whole blood sample, stand the centrifuge tube at room temperature (or 4°C). Take the supernatant for detection after all erythrocytes are settled to the bottom of the tube. For serum/plasma, sample can be used directly after mixed thoroughly.

2. Take the kit and sample out from cold storage and stand for 30 min to room temperature. Set the constant temperature box or water bath to 37 ± 1°C.

3. Preparation of washing buffer: Dilute the 20xConcentrated Wash Buffer with distilled water, purified water or deionized water at the ratio of 1:19, mix thoroughly.

4. Add sample: Reserve 1 well for positive control, 1 well for negative control (100 μL of corresponding control solution for each well). Set 1 well for blank control and add 100 μL of Sample Diluent. Add 100 μL of diluted sample (take supernatant of whole blood sample for detection) to other wells. Cover the plate with the plate sealer. Incubate for 60 min at 37°C. Carefully remove the plate sealer and discard the liquid in the wells. Add 300 μL of diluted washing buffer, gently shake for 10 sec and discard the liquid, tap the plate to make it dry. Repeat this washing process 5 times. Or operate this procedure with a plate-washer by setting the machine as the conditions above.

5. HRP conjugated: Add 100 μL of HRP Conjugate Working Solution to each well except blank control well. Cover the plate with sealer. Incubate for 30 min at 37°C. Carefully remove the plate sealer and discard the liquid in the wells. Add 300 μL of diluted washing buffer, gently shake for 10 sec and discard the liquid, tap the plate to make it dry. Repeat this washing process 5 times.

6. Add substrate: Add 50 μL of Substrate Reagent A and 50 μL of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37°C in the dark.

7. Stop reaction: Add 50 μL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
8. **OD Measurement**: Set the Micro-plate Reader wavelength at 450 nm/630 nm to detect the A value of each well within 30 min. Or set the Micro-plate Reader to zero with the blank well to determine the A value of each well directly when using single wavelength 450 nm for detection.

**Reference value**
1. Experimental result is valid if the positive control \(A_{450} < 0.3\), and the negative control \(A_{450} \geq 0.6\).
2. \[\text{Cut-Off (CO)} = \text{negative control (NC)} A_{450} \times 2.1, \frac{S}{CO} = \frac{\text{Sample} A_{450}}{\text{Cut-Off}}.\]
   
   \(0.9 \leq \frac{S}{CO} < 1.1\), the result is suspicious, and it is recommended to repeat the test.
   
   \(\frac{S}{CO} \geq 1.1\), the result is positive.
   
   \(\frac{S}{CO} < 0.9\), the result is negative.

**Interpretation of results**
1. **Positive result**: Varicella-zoster virus IgG in the sample is positive.
2. **Negative result**: Varicella-zoster virus IgG in the sample is negative.
3. **Suspicious result**: Varicella-zoster virus IgG in the sample is at critical value, it is recommended to do repeat detection or re-test after two weeks, and take dynamic observation of the change in antibody level.

**Product performance index**
1. **Coincidence of positive reference**: The detection result of 10 positive references were all positive.
2. **Coincidence of negative reference**: The detection result of 10 negative references were all negative.
3. **Sensitivity**: The detection result of sensitivity reference meet the required standard.
4. **Precision**: Detect 10 precision references, the CV% is less than 20%.
5. **Anti-interference performance**: Hemolysis with 2.0 mg/mL hemoglobin, blood lipid with 6 mmol/L triglyceride, and jaundice sample with 100 μmol/L has no interference for the results. Samples with auto-antibodies (rheumatoid factor, anti-nuclear antibody) and serum sample of pregnant woman will not interfere this kit.
6. **Cross reaction**: This kit has no cross reaction with positive samples of Measles virus IgG antibody, Rubella virus IgG antibody, Hepatitis A virus, Hepatitis B virus surface antibody, Bacillus tetani IgG antibody, Hepatitis C virus IgG antibody, Schaudinn's bacillus IgG antibody, HIV virus IgG antibody, Cytomegalovirus IgG antibody, Herpes simplex virus IgG antibody, Toxoplasma gondii IgG antibody. This kit has not been used with non-specific high IgG, non-specific high IgA and HAMA sample, clinical use should be avoided.
Notes
1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
2. The Stop Solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
3. The ELISA plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
4. 20× Concentrated Wash Buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should be kept fresh.
7. The results shall depend on the readings of the Micro-plate Reader.
8. Do not use components from different batches of kit.

Storage and Expiry date
1. This kit can be stored at 2~8℃ in the dark for 12 months. Prevent from freezing.
2. Unused pre-coated ELISA Micro-plate should be sealed in the valve bag with a desiccant immediately, and is valid for 1 week when stored at 2~8℃.
3. Other unused reagents should be covered immediately, and is valid for 1 week when stored at 2~8℃.