Porcine Circovirus Type 2 Antibodies ELISA Kit
Catalog No: E-AD-E003
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)    Fax: 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 kit is comprised by ELISA Microtiter plate pre-coated with recombinant cap protein of porcine circovirus II (PCV2), HRP conjugate and other auxiliary reagents and apply the principle of enzyme-linked immunoassay (ELISA) to detect porcine circovirus II antibody of porcine serum, plasma samples. During the experiment, add control serum and samples into the ELISA Microtiter plate. If PCV2 antibodies exist in the samples, it will be bound with the antigen pre-coated on the Microtiter plate after incubation. Then wash the plate to remove unbound antibodies and other components, add the HRP Conjugate to specifically bind with the compound of antibody and antigen on the Microtiter plate. The unbound HRP Conjugate will be removed by washing. TMB is added into the well, it will react with the enzyme and become blue. The color shade is of positive correlation with antibody levels in the samples. At last, end the reaction by adding stop solution to produce a yellow product. Measure the absorbance value of each well by using a Microtiter plate Reader with 450 nm wavelength, then the presence of PCV2 antibody can be determined.

Kit components

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Microtiter plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>Dilution plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>11 mL</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>50 mL</td>
</tr>
<tr>
<td>20×Concentrated Wash Buffer</td>
<td>40 mL</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1 piece</td>
</tr>
<tr>
<td>Manual</td>
<td>1 copy</td>
</tr>
</tbody>
</table>

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Experimental instrument
Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)
High-precision transferpettor, EP tubes and disposable pipette tips
37°C incubator or water bath
Deionized or distilled water
Absorbent paper
Sample preparation

1. Use the conventional method to prepare animal serum/plasma, the serum/plasma, must be clear, no hemolysis and no pollution. Samples can be conserved at 2~8°C in 1 week, and it should be stored at -20°C for a long term storage.

2. Dilute the sample serum/plasma with the sample diluent at 1:39 (5 μL sample serum/plasma and 195 μL of Sample Diluent, mix fully). The positive/negative control do not need to be diluted.

3. The 20xConcentrated Wash Buffer should be adjusted to room temperature before use, then dilute it with distilled or deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** Take out the Microtiter plate, set 1 well for blank control and 2 wells for negative/positive control respectively. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8°C. **Samples need test in duplicate.**

2. **Add sample:** Add 100 μL of Sample Diluent to the blank control well, add 100 μL of positive/negative control to positive/negative control well. Add 100 μL of diluted sample to the sample wells.

3. **Incubate:** Cover the plate sealer and mix thoroughly, incubate at 37°C for 30 min in the dark.

4. **Wash:** Remove the liquid in each well. Immediately add 300 μL of Wash Buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).

5. **HRP conjugate:** Add 100 μL of HRP conjugate into each well (except the blank control well). Cover the plate sealer and incubate at 37°C for 30 min in the dark.

6. **Wash:** Repeat Step 4 for washing.

7. **Color Development:** Add 50 μL of Substrate Reagent A and 50 μL of Substrate Reagent B into each well. Cover the plate sealer and incubate at 37°C for 10 min in the dark.

8. **Stop reaction:** Add 50 μL of Stop Solution into each well, mix thoroughly.

9. **OD Measurement:** Adjusted zero with the blank control, measure the absorbance value (A-value) of each well by using a Microtiter plate Reader with 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) wavelength. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection.
Reference value

Normally, the A-value of negative control ≤ 0.1 and the A-value of positive control ≥ 0.6.

Interpretation of the results

1. Positive result: $A_{450} ≥ 0.38$
2. Suspicious result: $0.38 > A_{450} > 0.2$
3. Negative result: $A_{450} < 0.2$
4. The negative result or suspicious result of this test suggests that the concentration of antibody for the tested porcine is not enough, it is recommended that this porcine should get immunized with corresponding vaccine.

Limitations of this test method

1. This test is only used as the qualitative detection of PCV2 antibodies in porcine serum and plasma. It can be used to evaluate the immune effect of pig blue ear virus vaccine. A rough estimate (high, general, low) of the concentration of this antibody according to the A values.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.
3. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
4. In the early stage of infection, antibody did not occur or has a low titer, and these situations will lead to negative results.
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. Concentrated washing liquid 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 Microtiter plate Reader.
8. Do not use components from different batches of kit.
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and Expiry date
Store at 2~8℃. Avoid freeze for 12 months.
Please store the opened plate at 2~8℃, protect from light and moisture. The valid period is 1 months.
Expiry date: expiration date is on the packing box.