

**PORCINE CIRCOVIRUS (PCV2) ELISA TEST  
KIT  
MANUAL**

## Porcine Circovirus (PCV2) ELISA Test Kit

Catalogue Number. IP100174

### **Product Usage**

The porcine circovirus(PCV2) ELISA Test kit is used for detection of porcine circovirus type 2 antibody in porcine serum; assessment of immunity conditions against porcine circovirus in the pig farm and investigation of the epidemiology of the porcine circovirus. While the result can not distinguish immune antibody and infectious antibody, to confirm that is antibody by vaccine or wild virus infection needs to combine clinical data, immune status, and other diagnostic methods.

### **Principle**

The PCV2 antibody ELISA test kit is made from the antigen coated microtiter plate(coated with PCV antigen) and other reagents. It applies the Solid-phase ELISA principle to PCV-Ab in serum, then add enzyme conjugate to specifically bind with complex of coated antigen+PCV-Ab+enzyme labeled anti-pig-IgG antibody on the microplate. With the TMB substrate, it will generate an amount of color. The depth of color is relative with the content of the PCV-Ab, when the value of color is greater than the cut-off value, the pigs are vaccinated well or natural infected exist.

### **Technical specifications**

96 wells × 2.

### **Components**

1	PCV- Antigen coated microplate	96T X 2	
2	PCV-IgG Negative control serum	1.5 mL/tube	green lid
3	PCV-IgG Positive control serum	1.5 mL/tube	red lid
3	Enzyme conjugate	22ML	yellow lid
4	20×concentrated washing solution	50ml	white lid
5	Substrate	1 bottle	orange lid
6	Stop solution	12ml	blue lid
7	Sample diluent solution	50 ml	transparent lid
8	Adhesive Foil	6 pieces	
9	Instruction	1 pieces	

### **Material required not provided**

- 1 Microplate Reader (Dual-wave length: 450/630 nm).
- 2 Microplate Washers.
- 3 Micropipettes, adjustable (Single-wave length 1-100ul、0.5-10ul、 multi-wave length 30-300ul).

- 4 Constant temperature box or water bath box.
- 5 Oscillators.
- 6 Disposable tips (10ul, 200ul)
- 7 Deionized water

### **Sample requirement**

- 1 The samples are porcine serum, which should be collected with no bacteria. The storage time should be less than 1 week at 2-8 °C, if for long term, it should be kept at -20°C.
- 2 Avoid to use the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
- 3 The EDTA, heparin sodium and other anticoagulants will not affect the results.

### **Preparation**

1. Bring ELISA reagents to the room temperature (20-25 °C) for 30 min to get best results. Microplate should return to room temperature and dry before open package.
2. Sample dilute: Dilute sample with the sample diluent at 40 times. (5ul serum + 195ul sample diluent), the diluted sample need to mix evenly to get better results.
3. Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water at 20 times. (50ml 20×concentrated washing buffer + 950ml deionized water ) It is normal if there is crystallization in the 20×concentrated washing buffer, put at 37°C until completely dissolved.

### **Operation procedures**

1. Take pre-coated microtiter strips (Can unseal for several time use as per sample quantity), add 100µL diluted serum to test well, meanwhile set 2 wells for Negative control, 2 wells for Positive control separately. Add 100 µL Negative/Positive control to its well. Shake softly, cover and incubate at 37°C for 30 min.
2. Pour the liquid out of the wells, add diluted washing solution to each well fully, be static for 10s, pour out. Wash 3 times, at last pat to dry on absorbent paper.
3. Add 100 µL Enzyme Conjugate to each well, and incubate at 37°C for 30 min.
4. Repeat the step 2(washing). Remember pat to dry on absorbent paper at last.
5. Add 100 µL substrate to each well, mix properly, react for 10 min at 37°C at dark.
6. Add 50 µL stop solution in each well, and measure the result within 10 min (shake evenly before read result, no spill).
7. Read OD value with ELISA reader at 450nm/630nm.

### **Results**

For the assay to be valid, the positive control wells' average OD value must be greater than or equal to 0.6, and the negative control wells' average OD value is less than 0.1. Otherwise the test is invalid, need test again.

The result is judged by S/P value,

$S/P = \frac{\text{Sample OD}_{450/630} - \text{NCx}(-)}{\text{PCx}(-) - \text{NCx}(-)}$ , NCx(-) means Negative control's average OD<sub>450/630</sub> value, PCx(-) means Positive control's average OD<sub>450/630</sub> value

If  $S/P \geq 0.2$ , it is positive; less than 0.2, it is negative.

### ***Interpretation of the result***

1. Severe hemolysis, fiber protein in the serum separation is not sufficient, containing erythrocytes, a precipitate, a sample with bacteria may lead to false positive.
2. Negative results may occur on individual pigs after vaccines due to individual differences or immune duration.
3. Positive results for serological diagnosis and epidemiological investigation of swine to be combined with other methods and clinical data.

### ***Product performance***

1. Specificity: use this kit to detect reference serum, the compliance rate reach 100%.
2. Sensitivity: can reach max 1:5120.
3. Precision: CV (%) no bigger than 8%.
4. Stability: Store at 2°C~8°C for 12 months or store at 37°C for 3 days, the result can reach the above 3 standard.

### ***Precautions***

1. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
2. Experiment rubbish should be dealt with high pressure steam sterilization at 121 °C for 30 minutes, or treated with 5.0g/L sodium hypochlorite disinfectant for 30 minutes, then discard.
3. Micro Well plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, and then can be opened. Put back unused MicroWell plate into dry foil bag and sealed at 4 °C. Unused liquid reagent should cover caps, store at 2-8 °C in dark with other group components.
4. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37°C until been dissolved.
5. Should use Micropipette to add sample and reagents, and often proof its accuracy.
6. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.

***Storage:*** store at 2-8°C, dark, sealed, dry place, no frozen.

***Expiry date:*** 12 months; date of production is on box.