

**PORCINE PSEUDORABIES VIRUS GB  
ANTIBODY ELISA TEST KIT  
MANUAL**

## Porcine Pseudo Rabies Virus GB Antibody ELISA Test Kit

**Catalogue Number. IP100171**

### ***Product Usage***

This kit is used to detect Porcine Pseudo rabies Virus GB antibody in porcine serum.

### ***Principle***

The Porcine Foot And Mouth Disease(FMD) IgG Type A antibody ELISA test kit is made from the antigen coated microtiter plate(coated with FMD-A-VP1 antigen) and other reagents. It applies the Solid-phase ELISA principle to FMD-A-VP1-Ab in serum, then add enzyme conjugate to specifically bind with complex of coated antigen+FMD-A-VP1-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 FMD-A-VP1-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	PRV-GB Antigen coated microplate	96T X 2
2	PRV-GB Negative control serum	1.5 mL/tube
3	PRV –GB Positive control serum	1.5 mL/tube
3	Enzyme conjugate	22ML
4	20×concentrated washing solution	50ml
5	Substrate A	1 bottle
6	Stop solution	12ml
7	Sample diluent solution	50 ml
8	Adhesive Foil	2 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. Sample dilute: Dilute sample with the sample diluent at 40 times.(3ul serum + 117ul sample diluent), the diluted sample need to mix evenly to get better results. Negative control and positive control do not need dilute.
2. Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water or distilled water at 20 times. (10ml 20×concentrated washing buffer + 190ml 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,

$S/P = (\text{Sample OD}_{450/630} - \text{NCx}(-)) / (\text{PCx}(-) - \text{NCx}(-))$ , 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.

### **Precautions**

1. All reagents should return to the room temperature (18~26°C) before using, and store back at 2-8°C after using
2. Do not use kit out of expiry date. Does not mix use reagents from kits of different lot numbers.
3. The unused micro-wells should seal back to bag and store back at 2-8°C.

4. Used materials should be treated innocuously, and be handled in accordance with local, regional and national regulations.
5. Avoid Substrate exposure to bright light, avoid contact with oxidants.
6. When the quantity of serum sample is big, dilute all serum sample on serum dilute plate firstly, then transfer all diluted serum sample on reaction wells, make the reaction time same.
7. Stop solution is corrosive, if splashed on the skin or clothing should immediately rinse with plenty of water.
8. Strictly adhere to instruction to get best result. All procedure including pipetting, timing and washing etc. must be accurate to get accurate result.

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

**Expiry date:** 24 months; date of production is on box.