

**PORCINE TOXOPLASMOSIS IGG  
ANTIBODY ELISA TEST KIT  
MANUAL**

## Porcine Toxoplasmosis IgG Antibody ELISA Test Kit

Catalogue Number. IP100166

### **Product Usage**

Porcine Toxoplasmosis IgG antibody test kit is used for the detection of the Toxoplasmosis antibodies in porcine serum.

### **Principle**

The test kit is enzyme immunoassay for the detection toxoplasmosis antibodies in porcine serum. Toxoplasmosis antigens are coated on 96-well plate, add sample into wells and then antibodies bound specifically by antigens, the unbound antibodies and other components will be removed by washing. Next add enzyme conjugation to specifically bind with the compound of antibodies and antigens on the microplate. The unbound enzyme conjugation will be removed by washing. Add the TMB substrate in the well, react to a blue product. At last, end the reaction by adding stop solution.

### **Technical specifications**

96 wells × 2.

### **Components**

1	TOX Antigen coated microplate	2 pieces
2	TOX-IgG Negative control serum	1.5 mL/tube
3	TOX-IgG Positive control serum	1.5 mL/tube
3	Enzyme conjugate	1 bottle
4	20×concentrated washing solution	1 bottle
5	Substrate A	1 bottle
6	Stop solution	1 bottle
7	Sample diluent solution	1 bottle
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.

### ***Operation procedures***

- 1 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 µL/well. Others are wells for samples, add 100µL/well of Sample diluent solution, then add 1 µL serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37°C for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 µL enzyme comjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 °C for 30 min.
6. Repeat step 3(washing).
7. Add 50µL substrate A and substrate 50µL B into each well, mix properly, incubate for 15 min at 37 °C in the dark with new adhesive foil.
8. Add 50µL stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

### ***Results***

Cut-Off Value (C.O.) = 0.35

Generally speaking, the TOX-IgG Positive control OD mean should be greater than or equal to 0.70, the TOX-IgG Negative control OD mean should be less than 0.10, otherwise the test is invalid, need to retest.

If the sample OD value  $\geq 0.35$ , the sample is classified as POSITIVE for TOX-IgG antibodies;

If If the sample OD value  $< 0.3$ , the sample is classified as NEGATIVE for TOX-IgG antibodies.

If between 0.3 and 0.35, it is skeptical

### ***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: to test 30 negative control serums, no false positive.
2. Sensitivity: to test 30 positive control serums, no negative.
3. Precision: CV (%) no bigger than 15% (n=10)
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 standards.

### ***Precautions***

This test kit is suitable for in vitro diagnostics.

2. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
3. 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.
4. 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.
5. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37°C until been dissolved.
6. Should use Micropipette to add sample and reagents, and often proof its accuracy.
7. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.
8. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

***Storage:*** store at 2-30°C, dark, sealed, dry place, no frozen.

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