

Porcine circovirus (Type 2) Ab ELISA test kit

**INSTRUCTION MANUAL
FOR PRODUCT No: LT507001BZ2**



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MANUAL VERSION 1.01

INTRODUCTION

The Porcine circovirus type 2 (PCV2) Ab Elisa kit is used to detect the antibodies to the circovirus type 2 in pig serum, and to evaluate the immune status of the PCV2 vaccine in pig farms and the serological diagnosis of infected pigs.

PRINCIPLE

This kit use indirect ELISA method, pured PCV antigen is pre-coated on micro-well strips. When testing, add diluted serum sample, after incubation, if there is PCV specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components with washing, then add enzyme labeled anti-PCV monoclonal antibody, antibody in sample block the combination of monoclonal antibody and pre-coated antigen, discard the uncombined enzyme conjugate by washing. Add TMB substrate in micro-wells, the blue signal by Enzyme catalysis is in inverse proportion of antibody content in sample, use ELISA reader at 450nm wavelength to measure the absorbance A value in reaction wells after adding stop solution to stop the reaction..

MATERIALS SUPPLIED WITH THIS KIT

1	Antigen coated microplate	96T×4	7	Stop solution	11 mlX2
2	Enzyme conjugate	22 ml X2	8	Washing solution 10X	100 mlX2
3	Sample diluent	100 mlX2	9	Adhesive Foil	8 pieces
4	Negative control	1.6 mlX2	10	Serum dilution plate	4 pieces
5	Positive control	1.6 mlX2	11	Instruction	1 piece
6	Substrate	22 mlX2			

MATERIALS REQUIRED BUT NOT PROVIDED

- 1) Micropipette: 0.5 μ l-10 μ l, 10 μ l-100 μ l, 100 μ l-1000 μ l.
- 2) Disposable pipette tips.
- 3) Cylinder: 500ml. 4) Microplate Reader: 96 wells.
- 5) Distilled water or deionized water. 6) Microplate Washer

SAMPLE PREPARATION

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

WASHING BUFFER PREPARATION

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4°C for about 1 month.

PROCEDURE

1) Take the antigen coated plate (the plate can be open and used for several times according to sample quantity each time), add the diluted serum to reaction wells, 100 μ l/well; meanwhile, set 2 wells for positive control and 1 well for negative control, both positive control and negative control do not need dilute, take 100 μ l directly and add into its well, mix gently (do not overflow).

2) Cover it with adhesive plate sealer, incubate at 37 °C for 30 minutes;

3) Open the adhesive plate sealer, discard the liquid of the well

Add diluted washing buffer to each well, 250 μ l/well, be static for 1min, then discard the liquid, repeat the above step for 4-6 times, at last flap to dry with the absorbent paper.

- 4) Adding Enzyme Conjugate, 100µl/well, Cover it with Adhesive plate sealer, incubate at 37 °C for 30 minutes;
- 5) Open the adhesive plate sealer, discard the liquid of the well, washing 4-6 times as step 3, remember at last flap to dry with the absorbent paper;
- 6) Add substrate: add Substrate 100µl/well, mix it evenly then cover it with Adhesive plate sealer, incubate at 37 °C in dark for 10 minutes;
- 7) Add stop solution 50µl/well to stop the reaction, measure the result in 10 minutes.

INTERPRETATION OF RESULTS

Read the OD value with ELISA Reader at 450nm (630nm as reference).

For the assay to be valid:

Negative control (N) OD value < 0.2, meanwhile positive control (P) OD value > 0.4.

Calculate method:

S/P value = Sample OD Value/Positive control OD average value

Results interpretation

S/P value < 0.5 Negative

S/P value ≥ 0.5 Positive

NOTE

- 1) Return all reagents into room temperature before use, shake it evenly before use, and store back to 2-8°C after usage.
- 2) Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.
- 3) Substrate and stop solution may have irritation to skin and eyes, be careful to

use.

- 4) Do not expose Substrate to strong light and avoid contact with the oxidant.
- 5) Pre-coated plates should be sealed and moisture-proof. Put back un-used Microwell plate into dry foil bag and sealed at 2-8 °C.
- 6) All wastes should be treated well to avoid pollution before discarding.
- 7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.
- 8) Antigen coated plate is disposable, do not re-use.

Specifications: 96 wells ×2.

Expiry date: 12 months.

Storage: at 2-8 °C, don't expose in strong light.

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PLEASE REFER TO MANUAL SUPPLIED WITH KIT FOR EXACT
DETAILS.**