Porcine Circovirus Type 2 Antibodies ELISA Kit

Catalog No: E-AD-E003

96T/96T*2

Version Number:	V1.5
Replace version:	V1.4
Revision Date:	2025.09.03

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit employs an indirect ELISA method, with purified PCV2 antigen pre-coated on the microtiter plate wells. During the experiment, diluted test serum is added. After incubation, if the sample contains PCV2-specific antibodies, they will bind to the PCV2 antigen on the coated plate. Following washing to remove unbound antibodies and other components, enzyme-labeled secondary antibody is added to specifically bind to the antigen-antibody complexes on the detection plate. After another wash to remove unbound enzyme-labeled substances, TMB substrate solution is added to the microplate wells, where it is catalyzed by the enzyme to produce a blue product. Finally, after adding the stop solution, the absorbance (OD value) in each reaction well is measured at a wavelength of 450 nm using a microplate reader.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
Dilution plate	96 wells
HRP Conjugate	12/12*2 mL
Sample Diluent	50/50*2 mL
10×Concentrated Wash Buffer	50/50*2 mL
Substrate Reagent A	6/6*2 mL
Substrate Reagent B	6/6*2 mL
Stop Solution	6/6*2 mL
Positive Control	800/800*2 μL
Negative Control	800/800*2 μL
Plate Sealer	3/6 pieces
Sealed Bag	1/2 piece
Manual	1 copy

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

Other materials required but not supplied

Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ incubator or water bath

Deionized or distilled water

Absorbent paper

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. 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 contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
4. Concentrated wash buffer 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 keep fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. **Each reagent is optimized for use in the E-AD-E003. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E003 with different lot numbers.**
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.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum:** Use the conventional method to prepare animal serum, the serum must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8℃ in 1 week, and it should be stored at -20℃ for a long term storage.
2. **Diluted serum:** Dilute the sample serum with the **Sample Diluent** at 1:101 (2 μL sample serum and 200 μL of sample diluent, mix fully). The positive/negative control do not need to be diluted.
3. **Wash Buffer:** The **10×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:9.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set up 2 well for the negative control, 2 well for the positive control and 1 well for the blank control.
2. **Add sample:** add 100 μL of **positive/negative control** to positive/negative control well, add 100 μL of sample diluent to blank control well, 100 μL of **Diluted serum** to the sample wells.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37℃ for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 350 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 3 times, 60 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. Cover the plate sealer and incubate at 37℃ for 30 min in shading light.
6. **Wash:** Repeat Step 4 for washing.
7. **Color Development:** add 50 μl of substrate reagent A solution, and then add 50 μl of substrate reagent B , mix well. Cover the plate sealer and incubate at 37℃ for 10 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) wavelength.

Reference value

Normally, the OD-value of negative control < 0.1 and the OD-value of positive control ≥ 0.4.

Interpretation of the results

1. Cut Off = 0.25+ average OD-value of negative control (when average OD_{450} of NC < 0.05, calculate at 0.05)
2. Positive result: Sample OD > Cut Off
3. Negative result: Sample OD < Cut Off

Limitations of this test method

1. This test is only used as the qualitative detection of PCV2 antibodies in serum of porcine.
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.