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LT70003AYSL

ELISA KIT FOR PORCINE CIRCOVIRUS 2

Research Use Only

For *in vitro* applications - not for consumption

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VERSION 1.2B

1.INTENDED USE

The Porcine Circovirus (PCV2) ELISA Test kit is used for detection of porcine circovirus type 2 antibody in porcine serum; assessment of immune 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 the is antibody produced due to vaccination or wild virus infection, combine clinical data, immune status, and other diagnostic methods.

2.KIT CONTENTS

- 1.PCV antigen coated microplate [96TX2]
- 2.Enzyme conjugate [22ml]
- 3.Sample Diluent Solution [50ml]
- 4.PCV-IgG Negative control serum [1.5ml]
- 5.PCV-IgG Positive control serum [1.5ml]
- 6.Substrate [12mlX2]
- 7.Stop Solution [12ml]
- 8.10X Washing Buffer [50ml]
- 9.Adhesive Foil [2pcs]
- 10.User manual [1pc]

Storage: Store at 2~8°C in dark, no freezing.

Expiry date: Mentioned on the box

3.MATERIALS REQUIRED NOT PROVIDED

Pipettes and Tips, ELISA Reader, Eppendorf tubes, Absorbent paper, Distilled Water, and 37°C Incubator.

4. SUMMARY AND PRINCIPLE

The PCV2 antibody ELISA test kit uses the principle of solid-phase enzyme-linked immunosorbent assay (ELISA), and consists of a microwell reaction plate coated with high-purity PCV2 antigen, anti-pig IgG labeled with horseradish peroxidase, and other reagents. The reaction mechanism is that the coated antigen is combined with the PCV-Ab in the sample, and then combined with the enzyme-labeled anti-pig IgG antibody to form a "coated antigen+PCV-Ab+enzyme-labeled anti-pig IgG antibody" complex. After adding the Substrate, catalytic reaction leads to color development. The color depth is directly proportional to the amount of PCV-Ab in the sample. The color intensity (Optical Density) is measured at dual-wave length 450nm/630nm.

5. SAMPLE REQUIREMENT

Porcine Serum - Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. If sediments occur during storage, centrifugation should be performed again.

Note:

1. Avoid using the samples with severe hemolysis, precipitated, or contaminated by bacteria or protein suspension.

2. The storage time should be less than 1 week at 2-8 °C, if for long term, it should be kept at -20°C.

6. PREPARATION

- 1) Bring ELISA reagents to the room temperature (20-25 °C) for 30 min to get best results. The micro-plate should be balanced at room temperature until the moisture dries out before it can be opened.
- 2) Sample dilution: Dilute sample with the sample diluent solution at 40 times (for example: 5µl serum +195µl sample diluent solution), the diluted sample needs to be mixed evenly to get better results.
- 3) Washing solution preparation: Dilute the 10×concentrated washing buffer with deionized water at 10 times (for example: 10ml 10×concentrated washing buffer+ 90ml deionized water), ready for use.

7. PROCEDURE

1. Take out the coated plates (Can be detached) and record the sample position on a worksheet.
Set 2 wells for negative control serum, add undiluted negative control serum, 2 wells for positive control serum, add undiluted positive control serum, 100µL/well (Not set blank well is OK). Others are sample wells, add the diluted sample, 100µL each.
2. Mix gently, cover with adhesive foil and incubate at 37°C for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add the diluted Washing buffer into each well fully, be static for 10 seconds, then pour out. Repeat 3 times, at last pat multiple times to dry on absorbent paper stack.
4. Add 100µL enzyme conjugate into each well.
5. Cover plate with new adhesive foil. Incubate at 37 °C for 30 min.

6.Repeat step 3(washing).

7.Add substrate 100ul into each well, mix properly, incubate for 10 min at 37 °C in the dark.

8.Add stop solution 50μL into each well, mix gently and determine the result.

9.Measure the OD value of each well with a photometer at dual-wave length 450nm/630nm.

8. RESULT & VALIDITY OF ASSAY

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, needs to be tested again.

9.CALCULATION AND RESULT INTERPRETATION

The result is interpreted by S/P value,

$S/P = (\text{Sample OD}_{450/630} - \text{NCx}(-)) / (\text{PCx}(-) - \text{NCx}(-))$,
NCx(-) means Negative control's average OD_{450/630} value,
PCx(-) means Positive control's average OD_{450/630} value
If $S/P \geq 0.2$, it is positive; less than 0.2, it is negative.

10. PRECAUTIONS

1. Do not use expired reagents, do not mix reagents from different lots.
2. Microwell plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, then can be opened. Put back unused MicroWell plate into dry foil bag, sealed and stored at 4 °C. Unused liquid reagent should be stored at 2-8 °C in dark with other group components.
3. Stop solution is corrosive, use large amount of water to wash immediately when touched the skin or clothes.
4. Experimental waste should be treated with high pressure steam sterilization at 121°C for 30 minutes, or with 5.0g/L sodium hypochlorite disinfectant for 30 minutes, then it should be discarded.

11. TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

Still if you have any concerns please feel free to write to us at customerservice@lifetechindia.com



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