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Porcine Parvovirus (PPV) antibody ELISA kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT90003AYSL

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MANUAL VERSION 1.01

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INTRODUCTION

The Porcine Parvovirus (PPV) antibody ELISA test kit is used for detection of Porcine Parvovirus (PPV) IgG antibody in porcine serum qualitatively, assessment of immunity conditions against porcine Parvovirus in the pig farm and investigation of the epidemiology of the porcine Parvovirus.

PRINCIPLE

The Porcine Parvovirus (PPV) antibody ELISA test kit is made from the antigen coated microtiter plate (coated with PPV antigen) and other reagents. It applies the Solid-phase ELISA principle to PPV-Ab in serum, then add enzyme conjugate to specifically bind with complex of coated antigen+PPV-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 PPV-Ab, when the value of color is greater than the cut-off value, the pigs are vaccinated well or natural infected exist.

MATERIALS SUPPLIED WITH THIS KIT

1	PPV- antigen coated microplate	96T×2	6	Substrate	12 ml orange lid
2	Enzyme conjugate	22 ml yellow lid	7	Stop solution	12 ml blue lid
3	Sample diluent	50 ml transparent lid	8	20X concentrated washing buffer	50 ml white lid
4	PPV-IgG Negative control serum	1.5 ml green lid	9	Adhesive Foil	2 pieces
5	PPV-IgG Positive control serum	1.5 ml red lid	10	Instruction	1 piece

MATERIALS REQUIRED BUT NOT PROVIDED

1) Microplate Reader (double-wave length: 450/630 nm).

2) Precise micropipette (single-channel 1-100µl, 0.5-10µl, multi-channel 30-300µl).

3) Constant temperature box or water bath.

4) Oscillator.

5) Microplate Washer.

6) Disposable tips (10µl, 200µl).

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 ℃, for long term storage, it should be kept at -20℃.

2. Avoid using sample of severe hemolysis, precipitate, contaminated by bacteria or protein suspension.

3. Samples with conventional dosage of EDTA, sodium citrate or sodium heparin a nticoagulant do not affect the experiment.

PREPARATION

1. Bring ELISA reagents to the room temperature (20-25 ℃) before 30 min prior to experiment to get best results.

2. Sample dilution: use the sample diluent at 40 times to dilute the sample. Mix the diluted sample evenly to get better results.

3. Washing solution preparation: Dilute the 20X concentrated washing buffer with deionized water at 20 times.

PROCEDURE

1. Take out the coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. 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 sample wells, add diluted sample, 100µl/well (both single-well and double-well test is OK).
2. Mix gently, cover with adhesive foil after adding sample and **incubate at 37 ℃ for 30 min.**
3. Remove adhesive foil. Pour the liquid out of the wells, add the diluted Washing solution into each well fully, be static for 10s, pour out directly. Repeat 3 times, at last time pat to dry on absorbent paper.
4. Add 100 µl enzyme conjugate into each well.
5. Cover with adhesive foil and **incubate at 37℃ for 30 min.**
6. Repeat step 3.
7. Add 100 µl substrate into each well, mix properly, **incubate for 10 min at 37 ℃ in the dark** with new adhesive foil.
8. Add 50 µl stop solution into each well, shake for 10s, and determine the result.
9. Read OD value of each well with ELISA Reader at double-wave length: 450/630 nm.

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, needs to be tested again.

The result is judged by S/P value,

S/P = (Sample OD450/630- $\overline{NC}$)/($\overline{PC}$- $\overline{NC}$), $\overline{NC}$ means Negative control's average OD450/630 value, $\overline{PC}$ means Positive control's average OD450/630 value

If S/P≥0.20, it is positive; less than 0.20, it is negative.

PRECAUTIONS AND WARNINGS FOR USERS

1. This test kit is suitable for *in vitro* diagnostics.
2. Read the Manual carefully before use.
3. Experiment rubbish should be dealt with high pressure steam sterilization at 121 ℃ 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, then can be opened. Put back unused Micro-well plate into dry foil bag and sealed at 4 ℃. Unused liquid reagent should cover caps, store at 2-8 ℃ in dark with other group components.
5. Should use Micropipettor to add sample and reagents, and often proof its accuracy.
6. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.

7. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

Specifications: 96 wells ×2.

Expiry date: 12 months.

Storage: Store at 2-8℃, in the dark.