VERSION 1.01

PORCINE PARVOVIRUS(PPV) ANTIBODY ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Porcine FMDV Type A antibody ELISA Kit

Catalogue Number. IP100176

Product Usage

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.

Technical specifications

96 wells × 2.

Components

1	PPV- Antigen coated microplate	96T X 2	
2	PPV-IgG-IgG Negative control serum	1.5 mL/tube	green lid
3	PPV-IgG Positive control serum	1.5 mL/tube	red lid
3	Enzyme conjugate	22ML	yellow lid
4	20×concentrated washing solution	50ml	white lid
5	Substrate A	12ml X2	orange lid
6	Stop solution	12ml	blue lid
7	Sample diluent solution	50 ml	transparent lid
8	Adhesive Foil	2pieces	
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 o r water bath box.
5 Oscillators.
6 Disposable tips (10ul, 200ul)
7 Deionized water

Preparation

1.Bring ELISA reagents to the room temperature (20-25 ℃) for 30 min to get best results. Microplate should return to room temperature and dry before open package.
2. Sample dilute: Dilute sample with the sample diluent at 40 times.(5ul serum + 195ul sample diluent), the diluted sample need to mix evenly to get better results.
3. Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water at 20 times. (50ml 20×concentrated washing buffer + 950ml deionized water) It is normal if there is crystallization in the 20×concentrated washing buffer, put at 37℃ until completely dissolved.

Operation procedures

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. Others are sample wells, add the diluted sample, 100μL/well.
2 Mix gently, cover and incubate at 37℃ 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 10s and pour out. Repeat 3 times, at last time pat to dry on absorbent paper.
4 Add 100μL enzyme conjugate into each well.
5 Cover plate with new adhesive foil. Mix gently, Incubate at 37 ℃ for 30 min.
6 Repeat step 3(washing).
7 Add substrate 100ul into each well, mix properly, incubate for 10 min at 37 ℃ in the dark with new adhesive foil.
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

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, need test again.
The result is judged by S/P value,
S/P=(Sample OD450/630- NCx(—))/(PCx(—)- NCx(—)), NCx(—) means Negative control's average OD450/630 value, PCx(—) means Positive control's average OD450/630 value
If S/P≥0.2, it is positive; less than 0.2, it is negative.

Product performance

1. Specificity: use this kit to detect reference serum, the compliance rate reach 100%.
2. Sensitivity: can reach max 1:5120.
3. Precision: CV（%）no bigger than 8%.
4. Stability: Store at 2℃～8℃ for 12 months or store at 37℃ for 3 days, the result can reach the above 3 standard.

Precautions

1. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
2. 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.
3. 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 ℃. Unused liquid reagent should cover caps, store at 2-8 ℃ in dark with other group components.
4. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37℃ until been dissolved.
5. Should use Micropipette 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.

Storage: store at 2-8℃, dark, sealed, dry place, no frozen.
Expiry date: 12 months; date of production is on box.