VERSION 1.01

PORCINE PSEUDORABIES VIRUS GE ANTIBODY DISTINGUISHING TEST KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Porcine Pseudo rabies Virus gE Antibody ELISA Kit

Catalogue Number. IP100172

Product Usage

Porcine Pseudo rabies Virus gE Antibody Distinguishing Test Kit is used for detection of antibody against Pseudorabies virus gE glycoprotein in porcine serum; it is used to distinguish and diagnostic Porcine Pseudorabies Virus gE lack Vaccine immune pig and naturally infected pig

Principle

The Porcine Pseudo rabies Virus gE Antibody Distinguishing Test Kit is made from the antigen coated microtiter plate(coated with PRV-gE antigen) and other reagents. It applies the Solid-phase ELISA principle to PRV-gE-Ab in serum, then add enzyme conjugate to specifically bind with complex of coated antigen+PRVgE-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 PRV-gE-Ab, when the value of color is greater than the cut-off value, there is wild virus antibody.

Technical specifications

96 wells × 2.

Components

1	PRV-gE Antigen coated microplate	96T X 2
2	PRV-gEIgG Negative control serum	1.5 mL/tube
3	PRV-gE IgG Positive control serum	1.5 mL/tube
3	Enzyme conjugate	22ML
4	20×concentrated washing solution	50ml
5	Substrate A	1 bottle
6	Stop solution	12ml
7	Sample diluent solution	50 ml
8	Adhesive Foil	6 pieces
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

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 ℃, if for long term, it should be kept at -20℃.
2 Avoid to use the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
3 The EDTA, heparin sodium and other anticoagulants will not affect the results.

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 pre-coated microtiter strips (Can unseal for several time use as per sample quantity), add 100μL diluted serum to test well, meanwhile set 2 wells for Negative control, 2 wells for Positive control separately. Add 100 μL Negative/Positive control to its well. Shake softly, cover and incubate at 37℃ for 30 min.
2.Pour the liquid out of the wells, add diluted washing solution to each well fully, be static for 10s, pour out. Wash3 times, at last pat to dry on absorbent paper.
3. Add 100 μL Enzyme Conjugate to each well, and incubate at 37℃ for 30 min.
4. Repeat the step 2(washing). Remember pat to dry on absorbent paper at last.
5. Add 100 μL substrate to each well, mix properly, react for 10 min at 37℃ at dark.
6. Add 50 μL stop solution in each well, and measure the result within 10 min(shake evenly before read result, no spill).
7. Read OD value with ELISA reader at 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.

Precautions

1. All reagents should return to the room temperature (18~26℃) before using, and store back at 2-8℃ after using
2. Do not use kit out of expiry date. Does not mix use reagents from kits of different lot numbers.

3. The unused micro-wells should seal back to bag and store back at 2-8℃.
4. Used materials should be treated innocuously, and be handled in accordance with local, regional and national regulations.
5. Avoid Substrate exposure to bright light, avoid contact with oxidants.
6. When the quantity of serum sample is big, dilute all serum sample on serum dilute plate firstly, then transfer all diluted serum sample on reaction wells, make the reaction time same.
7. Stop solution is corrosive, if splashed on the skin or clothing should immediately rinse with plenty of water.
8. Strictly adhere to instruction to get best result. All procedure including pipetting, timing and washing etc. must be accurate to get accurate result.

Storage: store at 2-8℃, dark, sealed, dry place, no frozen.
Expiry date: 24 months; date of production is on box.