480Tests/kit

ELISA

Porcine Reproductive & Respiratory Syndrome Virus Antibody ELISA

AniGen PRRS Ab ELISA 4.0

Manufactured by BIONOTE
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Porcine Reproductive & Respiratory Syndrome Virus Antibody ELISA

1. Explanation of the Test

Porcine Reproductive and Respiratory Syndrome (PRRS) is considered the most economically important viral disease of intensive swine farms in Europe and North America. The disease may also be referred to as Swine Infertility and Respiratory Syndrome (SIRS) by some veterinarians and swine industry professionals. The syndrome first began causing swine herd problems in the late 1980's and, prior to isolation of the causative agent, was often referred to as mystery swine disease.

The most commonly used serological tests for PRRS diagnosis are the indirect fluorescent antibody (IFA) test and enzyme linked immunosorbent assay (ELISA).

The AniGen PRRSV Ab ELISA contains a microplate, which is pre-coated with recombinant PRRSV antigen in the well. For testing, ELISA plates coated with the antigen are incubated with diluted serum and then with anti swine IgG-HRP conjugate. Following this incubation, all unbound material is removed by aspiration and washing before the addition of a substrate solution. The residual enzyme activity found in the well will thus be directly proportional to the anti-PRRSV antibodies in serum. The reaction is stopped by addition of a stop solution and colorimetric reading will be performed using a spectrophotometer at 450nm with referance wavelength at 620nm.

The specially selected PRRSV antigens are used as capture material in the test. These enable the AniGen PRRSV Ab ELISA to identify PRRSV antibodies in serum and plasma, with a high degree of accuracy.

2. Sample Recommendation

For routine serologic herd monitoring, it is suggested that at least 10 or more sera per herd are randomly collected at standard time intervals (i.e. every four weeks). For proper sample collection procedures, serum harvest and serum samples should be stored (4°C for up to four days or -20°C for longer periods) and are needed to provide reliable test results. To achieve better specificity and to minimize possible false positive reactions, serum samples that are contaminated with bacteria or are very fatty should be excluded from testing.

3. Provided Materials (480 Wells/Kit)

AniGen PRRSV Ab ELISA contains the following items to perform the assay.

1) Antigen coated micro-assay plate (1) : 96 wells/plate x 5 plates, configured in twelve 1x8 strips.
2) Negative Control (2): 1 vial (0.5㎖) of SPF piglet serum preserved in phosphate buffer with protein stabilizer. Proclin 300 (0.05%) added as preservatives.
3) Positive Control (3): 1 vial (0.5㎖) of antibodies to PRRSV preserved in phosphate buffer with protein stabilizer . Proclin 300 (0.05%) added as preservatives.
4) Sample Diluent (4): 1 bottle (250㎖) of phosphate buffer, sodium azide (0.01%) added as a preservative.
5) Washing solution (20X concentrated) (5): 1 bottle (250㎖) of PBS-Tween 20. Preservative is Proclin 300 (0.05%).
6) Enzyme Conjugate (6): 1 bottle (80㎖) of rabbit anti-swine IgG HRP, BSA and stabilizers. Preservative is Proclin 300 (0.05%). Ready to use.
7) Substrate (7): 1 bottle (60㎖) of tetramethyl-benzidine with citrate-phosphate buffer : STORE IN THE DARK.

8) Stop solution(8): 1 bottle (80㎖) of 1N sulfuric acid. Ready to use.
9) Adhesive plate sealer.
10) Instructions for use.

4. Not Provided Materials

1) Dilution microplate or disposable test tube
2) Micro pipette
3) ELISA Washer
4) ELISA Reader

5. Precautions

In order to obtain reproducible results, the following must be observed :

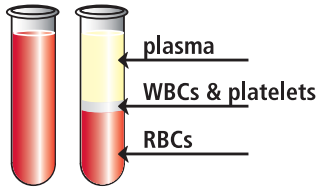
1) For in vitro diagnostic use only.
2) Do not mix reagent of different lots.
3) Use thoroughly cleaned glassware, free from contamination of metal ion or oxidating substances.
4) Use disposable gloves while handling potentially infectious material and performing the assay.
5) Substrate and stop solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident, rinse thoroughly with running water.

6. Specimen Collection and Storage

1) AniGen PRRSV Ab ELISA Test has been evaluated with swine samples only. Samples from other animals have not been evaluated.
2) Fresh serum or plasma samples should be used with this assay.
3) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to be used with the assay.

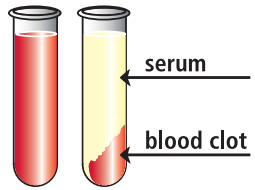
[Preparing of plasma samples]

(1) Blood should be collected with a disposable syringe and added to a tube containing anticoagulant (Heparin, EDTA, or Citrate), and then separate plasma by centrifugation.
(2) Plasma may be stored at 2~8°C for up to 2 weeks, for longer storage (1 year) freeze at below -20°C.



[Preparing of serum]

(1) Blood should be collected with a disposable syringe and added to a serum collection tube (no anticoagulant).
(2) Collected blood should be left at room temperature for 30 minutes to coagulate, and then separate serum by centrifugation.
(3) Serum may be stored at 2~8°C for up to 2 weeks, for longer storage (1 year) freeze at or below -20°C.



7. Reagent preparation

1) Allow all reagents to come to room temperature before use.
2) Washing solution (20X concentrated) : The concentrated washing solution (5) must be diluted 1 to 19 using distilled/deionized water before use.

For example) Mix 100㎖ of washing solution (20x concentrated) with 1,900㎖ of distilled/ deionized water. 500ml of working washing solution is sufficient for about 96 test wells.

8. Test procedure

Simple procedure

1) Prepare antigen coated micro assay plate
2) Dilute test samples and controls with sample diluent (1:39 dilution)
3) Add 100㎕ of diluted samples and controls to wells.
4) Incubate plate for 30minutes at room temperature (18~25°C).
5) Wash plate 5 times using the diluted washing solution.
6) Add 100㎕ of enzyme conjugate to wells.
7) Incubate plate for 30minutes at room temperature (18~25°C).
8) Wash plate 5 times using the diluted washing solution.
9) Add 100㎕ of substrate and incubate for 15minutes at room temperature in the dark.
10) Add 100㎕ of stop solution.
11) Measure the optical density (OD) at 450nm with reference wavelength at 620nm.

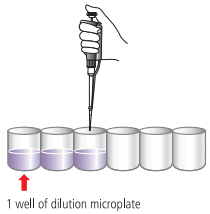
Detail procedure with picture

Sample dilution procedure

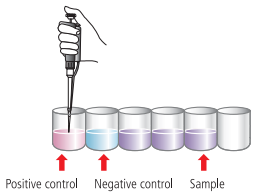
1) Use the micro plate or test tube for dilution (Not provided)

Or

2) Add 390㎕ of sample diluent into each well/tube



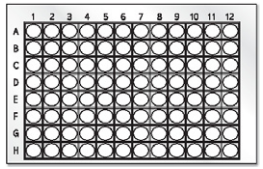
3) Add 10㎕ of negative control, 10㎕ of positive control and 10㎕ of sample to be tested into separate wells/tubes



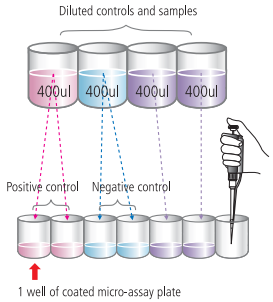
Test procedure

1) Use the antigen coated micro-assay plate (Provided)

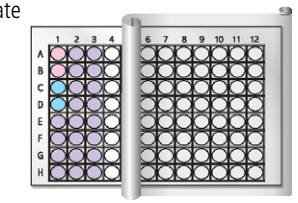
[1 plate]



2) Add 100㎕ of diluted positive control to two (2) wells, 100㎕ of negative control to two (2) wells and each samples to be tested to a separate well to antigen coated micro-assay plate

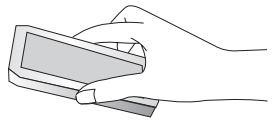


3) Cover the plate with the adhesive plate sealer and incubate at room temperature (18~25°C) for 30±1 minute

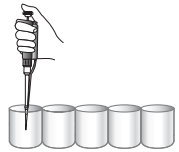


4) Wash plate 5 times using ELISA washer or micropipette

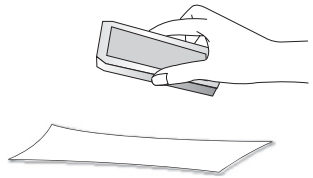
① Remove controls and samples



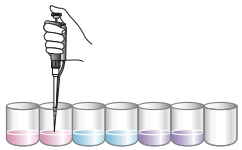
② Add 300㎕ of diluted washing solution and remove it. Repeat this procedure 5 times



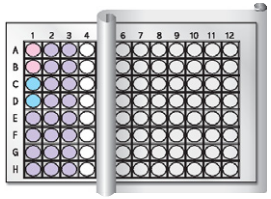
③ Remove diluted washing solution by tapping thoroughly on absorbent paper towel



5) Add 100μl of Enzyme conjugate into each well

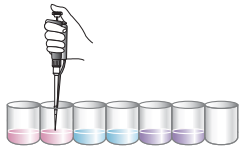


6) Cover the plate with the adhesive plate sealer and incubate at room temperature (18~25°C) for 30±1 minute

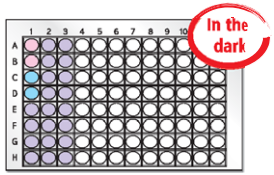


7) Wash plate 5 times using ELISA washer or micro pipette [Repeat steps 4]

8) Add 100μl of Substrate into each well



9) Incubate at room Temperature (18~25°C) for 15 minutes in the dark.



10) Add the 100μl of stop solution into each well

11) Measure the optical density (OD) at 450nm with reference wavelength at 620nm.

9. Interpretation of the Test

1) Test validation

① If the OD450 of a test sample is less than the mean OD450 negative, the S/P ratio can be interpreted as 0.

② The absorbance of the positive control mean (PCx) should be above 0.250. If these specifications are not met, the test must be repeated. The negative control mean (NCx) must be less than or equal to 0.200

2) Results calculation

① Calculation of NC mean: $\frac{NC1 + NC2}{2}$

② Calculation of PC mean: $\frac{PC1 + PC2}{2}$

③ Criteria: The criteria is based on following formula.

$$S/P\ ratio = \frac{(OD_{450}\ sample - OD_{450}\ mean\ NCx)}{(OD_{450}\ mean\ PCx - OD_{450}\ mean\ NCx)}$$

3) *Positive*: If the S/P ratio is greater than or equal to 0.4, the sample is regarded as positive for PRRSV antibodies.

4) *Negative*: If the S/P ratio is lesser than 0.4, the sample is regarded as negative for PRRSV antibodies.

(For example)

- Mean OD negative : 0.112
- Mean OD positive : 0.514
- OD sample: 0.324

$$S/P\ ratio = \frac{(0.324 - 0.112)}{(0.514 - 0.112)} = \frac{0.212}{0.402} = 0.53$$

This sample is classified as **positive** for PRRSV antibodies.

10. Limitations and Interferences

1) Test procedure, precautions and interpretation of results sections for this test kit must be followed when testing.

2) Samples

① Samples containing sodium azide do not affect the test result.

② Pasteurized samples (no less than 10 hours at 60℃) may lead to diminished reactivity and therefore should not be used.

③ Heat-inactivated samples (30 minutes at 56℃) do not impair the test.

④ Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.

⑤ Hemolytic samples should be centrifuged before use to avoid interference by cellular constituents.
⑥ Lipaemic and icteric samples do not impair the test results.

3) Failure to add specimen to the test well could result in a false negative test. Repeat testing should be considered where there is clinical suspicion of infection.

11. Storage

The kit should be stored at 2~8℃. This test kit is stable through the expiration date printed on the package and on the label of each material / reagent in an unopened state.

12. Stability

1) Do not use after the stated expiry date.
2) Stability of prepared reagents

Material / reagent	State	Storage	Stability
Working Washing Solution	Once opened	Room Temp.	1 week

13. Trouble shooting

If the test does not perform satisfactorily, check the test procedure instructions were carried out correctly

1) No color after 30 minutes incubation
   - Enzyme conjugate contaminated
   - Enzyme conjugate was not dispensed into sample well
2) Color develops too slowly
   - After washing plate, the plate became dried out.
   - Stop solution was added to test instead of substrate
   - The substrate was not allowed to come to room temperature before use.
3) Color develops too quickly
   - Poor washing
   - Enzyme conjugate contaminated
4) All wells are colored
   - Poor washing
   - Substrate contaminated
5) Patchy or poor color
   - Poor pipetting or washing
   - Poor mixing of reagents
   - Dirty glassware

14. Packaging Unit

96 Tests/kit, 480 Tests/kit, 960 Tests/kit

15. Precision

Within-run and between-run precisions have been determined by testing 10 replicates of three specimens: a negative serum, a low positive serum and a strong positive serum. The C.V (%) of negative, low positive and strong positive values are within 10%.

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