VERSION 1.01

ACTINOBACILLUS PLEUROPNEUMONIAE(APP)APXIV ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Actinobacillus Pleuropneumoniae(APP) ApxIV Antibody ELISA Kit For Swine

Catalogue Number. IP100184

Usage and Principle

This kit use indirect enzyme-linked immunoassay (indirect ELISA) to detect specific Actinobacillus Pleuropneumoniae(APP) ApxIV antibody in swine serum or plasma. Applicable to the differential diagnosis of Actinobacillus Pleuropneumoniae infected pigs and inactivated vaccines or subunit vaccines Immune pigs.
If there is Actinobacillus Pleuropneumoniae(APP) ApxIV antibody in sample, it will combine with coated Actinobacillus Pleuropneumoniae(APP) ApxIV antigen on plate and enzyme conjugate added on second step. The color reaction of anti-porcine enzyme conjugate on plate with substrate will reflect whether the sample is infected with Actinobacillus Pleuropneumoniae(APP)

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	ApxIV-Ag Coated plates	96 wells*2
2	Enzyme Conjugate	22 ml
3	10X Washing solution	100 ml
4	Substrate A	15 ml
5	Substrate B	15 ml
6	Sample dilution	100 ml
7	Stop solution	15 ml
8	Positive control	1ml
9	Negative control	1ml
10	Adhesive Foil	6 pieces
11	Instruction sheet	1 piece
	Serum dilution plate	2 piece

Material required not provided

1. Microplate Reader with 450nm and 630nm
2) 37 ℃ thermostatic device
3) Micropipettes, adjustable

Sample requirement

1) This kit only used to testing porcine serum or plasma.
2) To get the best test result, avoid using sample with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
3) The serum sample can store at 2-8 ℃ for short term (in 3 days), if for long term, it should be kept at -20℃ or lower, avoid repeated freezing and thawing.

Operation procedures

1) Washing Solution preparation: Dilute 10X Washing solution with distilled water or deionized water at 1:10 (for example: take 100ml 10X Washing solution, add 900ml distilled water or deionized water, mix),mix it evenly to get washing solution.
2) Sample dilute and adding sample: Dilute serum sample with sample dilution at 1:40, add only diluted serum sample to sample wells, 100ul/well. If use Serum dilution plate to dilute sample, the mothed is as following: add 195ul Sample dilution into Sample dilution plate, then add 5ul serum, use Pipettes to blow and stir to mix it evenly, then take 100ul solution into the sample wells. (Note:the Serum dilution plate is disposable, do not repeat use.)
3) Adding controls: Negative control and positive control do not need dilute, add it directly. Set 2 wells for blank control, only add 100ul sample dilution to each well; 2 wells for negative control, only add 100ul negative control to each well; 2 wells for Positive control, only add 100ul Positive control to each well. Cover plate with adhesive foil, incubate in dark at 37 ℃ for 30 minutes.
4) Washing plate: discard the liquid of the well, fill full (add 250ul/well if using washing machine)with washing solution to each well, washing for 3 times, be static for 1 min for each time; at last time, flap to dry with the absorbent paper.
5) Adding Enzyme Conjugate: Except blank control, add Enzyme Conjugate to each well, 100ul/well, Cover plate with adhesive foil, incubate in dark at 37 ℃ for 30 minutes. Discard liquid of the wells and wash 3 times as described in step 4).
6). Adding substrates: according to the quantity needed, take same volume of Substrate A and Substrate B, mix them evenly before use, then add into all well,100ul/well, Cover plate with adhesive foil, incubate in dark at 37℃ for 10 minutes.
7) Stop reaction: Add 50ul Stop solution into all wells to stop reaction. Set zero at blank control, read the OD value at ELISA reader 450nm (630nm as a reference).

Results

S/P= OD value of sample/ Average OD value of Positive control
S/P value ≥ 0.2: Actinobacillus Pleuropneumoniae(APP) ApxIV antibiody Positive;
S/P value < 0.2: Actinobacillus Pleuropneumoniae(APP) ApxIV antibiody Negative

Notes

1.*Micro Well 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 and sealed at 2~8 ℃.*
2. Before adding reagent, gently shake dropping bottle and mix even the reagent.
3.When incubation, must cover the plate with adhesive foil, do not repeat use the adhesive foil.
4.Don't mix use components and instruction from different kits
5.The test kit and any samples should be regarded as the source of infection to properly handle. Stop solution is corrosive, be careful to use.

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