VERSION 1.01

PORCINE EPIDEMIC DIARRHEA(PED) ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Porcine Epidemic Diarrhea (PED) Antibody ELISA Kit

Catalogue Number. IP100182

Product Usage

This kit is used to detect Porcine Epidemic Diarrhea (PED) antibody in porcine serum.

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**	
1	PED-Coated plates 96 wells	96T X 2	
2	Enzyme Conjugate	11/22 ml	yellow lid
3	20X Concentrated washing buffer	100 ml	white lid
4	Substrate	11/22 ml	orange lid
5	Sample diluent	100 ml	transparent lid
6	Stop solution	12 ml	blue lid
7	Negative control	1.5 ml	green lid
8	Positive control	1.5ml	red lid
9	Adhesive Foil	2pieces	
10	Instruction sheet	1 piece	

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Washing buffer preparation

Return 20X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 20 times with distilled water or deionized water. The diluted washing buffer can store at 4℃ for about 1 week.

Sample dilution

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. Negative control and positive control do not need dilute.

Operation procedures

1 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. 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 wells for samples, add 100µL/well of Sample diluent solution, then add 1 µL serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37℃ for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 µL enzyme comjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 ℃ for 30 min.
6. Repeat step 3(washing).
7. Add 50µL substrate A and substrate 50µL B into each well, mix properly, incubate for 15 min at 37 ℃ in the dark with new adhesive foil.
8. Add 50µL stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

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(calculate as 0.05 when the value is less than 0.05), 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

All reagents should be adjusted to the room temperature and shake evenly before using, store at 2-8 ℃ after using
2) Do not exchange the reagents from the kits of different lot numbers to use. Avoid reagent pollution when using.
3) Substrate and stop solution may have excitant to skin and eyes, pay attention when using.
4) Do not expose TMB (Substrate) to light and avoid it contact with antioxidants.
5) The wells should avoid damp or touching water after unsealing (Put the un-using microtiter strips back to bag with dehydrator in 2~8 ℃ soon)
6) Deal all waste reasonable before dumping to avoid pollution.
7) Strictly adhere to instruction to get best result. All procedure including pipetting, timing and washing etc. must be accurate.
8) Serum dilution plate is disposable, do not use for second time; the MAX volume of it is 300μL/well.

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