VERSION 1.01

PORCINE FOOT-AND-MOUTH DISEASE VIRUS 3ABC ANTIBODY ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Porcine Foot-and-Mouth Disease Virus 3ABC Antibody ELISA Test Kit

Catalogue Number. IP100168

Product Usage

Porcine Foot-and-Mouth Disease Virus 3ABC ELISA Test Kit is used for detection of FMD non-structural 3ABC-IgG antibody in porcine serum qualitatively; it is used to distinguish Porcine FMD antibody between wild virus infection and produced by inactivated vaccine.

Principle

Porcine Foot-and-Mouth Disease Virus 3ABC ELISA Test Kit is made from the antigen coated microtiter plate(coated with 3ABC antigen) and other reagents. It applies the Solid-phase ELISA principle to FMD-3ABC-Ab in serum, then add enzyme conjugate to specifically bind with complex of coated antigen+FMD-3ABC-Ab+enzyme labeled anti-pig-IgG antibody on the microplate. With the TMB substrate, it will generate an amount of color. Read the OD value with ELISA reader at 450nm/630nm after stop reaction, the OD value will reflect the level of 3ABC antibody in porcine serum.

Technical specifications

96 wells × 2.

Components

1	FMD -3ABCAntigen coated microplate	96T X 2
2	FMD-IgG Negative control serum	1.5 mL/tube
3	FMD-3ABC-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.

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 0.15. 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≥0.3, it is positive; less than 0.3, it is negative.

Product performance

1. Specificity: to test 30 negative control serums, no false positive.
2. Sensitivity: to test 30 positive control serums, no negative.
3. Precision: CV（%）no bigger than 15%（n=10）

4. Stability: Store at 2℃～8℃ for 12 months or store at 37℃ for 3 days, the result can reach the above 3 standards.

Precautions

1.This test kit is for research use only.
2. Wear glove and working clothes when operate, treat the test kit as containing infectious material.
3. 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.
4. 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.
5. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37℃ until been dissolved.
6. Should use Micropipette to add sample and reagents, and often proof its accuracy.
7. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.
8. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

Storage: store at 2-30℃, dark, sealed, dry place, no frozen.
Expiry date: 24 months; date of production is on box.