LSY-30048 African Swine Fever Virus Antibody Blocking ELISA (b-ELISA) Kit

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Catalog No. LSY-30048

1. Brief and Usage

African swine fever (ASF) is a severe, acute, highly contagious infection of pigs caused by African swine fever virus (ASFV). ASF is characterized by high fever, cyanosis of the skin, severe bleeding of internal organs, respiratory disorders, neurological symptoms, short course of illness and high mortality. This kit based on VP72 antigen expression and specificity monoclonal antibody (McAb), adopting blocking enzyme-linked immunosorbent assay (b-ELISA), equipped with a full range of diagnostic reagents and materials, one box can detect 92 pieces of serum (or partial) according to the circumstance, can be used in African swine fever antibody detection, high sensitivity, strong specificity, repeatability, and the characteristics of the kit itself without infectivity.

Blocking enzyme-linked immunosorbent assay(b- ELISA) for specific African swine fever virus antibodies detection in swine serum samples.

2. Principle

The specific antibodies in the serum to be tested block the binding of ASFV and McAb. Add the serum to be tested into the 96 well enzyme plate hole coated with ASFV recombinant protein antigen. If there is specific antibody of ASFV in the serum to be tested, it will bind with the specific coated antigen. After blocking, add the binding reaction of enzyme-specific monoclonal antibody and antigen. The unbound enzyme-specific monoclonal antibody will be washed away and no color will appear after adding the substrate. On the contrary, if there is no specific antibody of ASFV in the serum to be tested, there will be color development after the substrate is added. The OD value of the reactant was measured by enzyme labeling instrument, the blocking rate was calculated, and the test results were determined.

3.The kit components

1	ASFV recombinant antigen coated microplate	96T	
2	ASFVmonoclonal Enzyme conjugate	12ml	yellow lid

3	ASFVSample diluent solution	50ml	transparent lid
4	ASFVNegativecontrol serum	0.5ml	green lid
5	ASFVPositivecontrol serum	0.5ml	red lid
6	ASFVSubstrate	12ml	orange lid
7	ASFVStop solution	7ml	blue lid
8	ASFV 10×concentrated washing buffer	12ml	white lid
9	Adhesive Foil	1 piece	
10	Instruction	1 piece	

4. Preparation

1) Bring ELISA reagents to the room temperature (20-25 ℃) for at least 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 solution at 10 times.(10ul serum + 90ul sample diluent solution), the diluted sample need to mix evenly to get better results. Negative and positive control do not need dilute, add it directly for testing.

3) Washing buffer preparation: Dilute the 10×concentrated washing buffer with deionized water at 10 times. (For example: 1ml 20×concentrated washing buffer + 9ml deionized water) Make the crystals completely dissolved before use. The diluted washing buffer can store for 7-10 days at 2~8℃.

5. Procedure

1. Take out the coated plates (Can be detached) and record the sample position on a worksheet. Set 2 wells for negative control serum, add undiluted negative control serum, 2 wells for positive control serum, add undiluted positive control serum, 100μL/well. Others are sample wells, add the diluted sample, 100μL each(Both single-well and double-well test is OK).
2. Mix gently, cover andincubate at 37℃ wet box for60 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add the diluted Washing buffer into each well fully, then pour out directly. Repeat 6 times, at last time pat to dry on absorbent paper.
4 . Add 100μL ASFV monoclonal Enzyme conjugate into each well, mix it gently.

5. Cover plate with adhesive foil.Incubate at 37 ℃ wet boxfor45 min.

6. Repeat step 3(washing).
7. Add substrate 100ul into each well, mix properly,incubate for 10 min at37 ℃ in the dark.
8. Add stop solution 50μL into each well, mix gently and determine the result.
9. Measure the OD value of each well with a photometer at dual-wave length 450nm/630nm.

6. Results

For the assay to be valid, the OD value of Negative control wells must be greater than or equal to 1.0, and the OD value of Positive control wells is less than 0.5. Otherwise the test is invalid, need test again.

Calculate the blocking rate(%) as following:

Blocking rate(%)=[(Average OD value of Negative control- Average OD value of Sample)/Average OD value of Negative control]x100%

If Blocking rate(%)≥50%: it is ASFV antibody positive;

Blocking rate(%)<45%: it is ASFV antibody negative;

45%≤Blocking rate(%)<50%, the serum sample is suspicious of ASFV antibody, it needs to recheck; retest the serum sample, if result still 45%≤Blocking rate(%)<50%, then judge it as ASFV antibody Positive.

7. Precautions and warnings for users

1). This kit is a pilot product. Batch number and expiry date see the label on box and instruction,do not use kit out of date.
2). The kit should store at 2~8℃, the shelf life is 12 months;
3). Read the Manual carefully before use.
4). All components in the kit need to put at room temperature for 30min before use.
5). The coated plate is a detachable ELISA plate, which can be disassembled and used for many times. The remaining coated strip shall be sealed to avoid moisture.
6). After the sample is added, it must be mixed evenly. Each sample should use a new pipette tip to avoid cross contamination.
7). Wash thoroughly.

8). The sensing time shall be strictly controlled and each step of operation shall be compact.
9). After termination, OD value shall be measured within 30min to avoid color change.
10). The serum to be tested can be diluted one day before the experiment and placed overnight at 4℃.
11). Do not expose the substrate to strong light and avoid contact with oxidant.

Specifications:96 wells.

Expiry date: 12 months.
Storage: Store at 2~8℃, in the dark, no frozen.
Production Date: On outer-packing of the test kit, use the kit during expiry date.