Technical Manual

Brucellosis Antibodies ELISA Kit

- Catalogue Code: AEES00743
- Competitive ELISA Kit
- Research Use Only

1. Description and Principle

The Assay Genie Brucellosis Antibodies ELISA Kit can assay for the specific antibodies in serum of the following species: cattle, sheep, swine and goat.

How Do Our ELISA Kit Assays Work?

This kit is based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with the Brucellosis (BC) antigen. During the reaction, BC antibodies will compete with HRP Conjugate to bind with the antigen pre-coated on the ELISA Microtiter plate. The unbound HRP conjugate will be removed by washing, and substrate reagent is added for color development. The absorbance value of each well is measured using a microplate reader with 450 nm (630 nm) wavelength. There is a negative correlation between the OD value of samples and the concentration of BC-Ab in the sample. The kit provides a positive and negative control.

2. Key features and Sample Types

Sample type: serum

Sample type: cattle, sheep, swine, goat

ELISA Type: Competitive

Specificity: This kit recognizes Brucellosis antibodies.

SUMMARY

1. Add samples, positive control and negative control to the wells.
2. Add Antibody Working Solution and HRP conjugate to the wells. Incubate for 90 min at 25°C.
3. Wash 5 times.
4. Add Substrate Reagent. Incubate for 15 minutes at 25°C in shading light.
5. Add Stop Solution. Read at 450 nm.
6. Calculation of results

3. Kit Contents

Product	Size	Cat. Code
Brucellosis Antibodies ELISA Kit	**96 assays**	**AEES00743**

Each kit contains reagents for 96 assays including:

Item	96T
Micro ELISA Plate	96 wells
Antibody Working Solution	3 mL
HRP Conjugate	3 mL
Sample Diluent	100 mL
Concentrated Wash Buffer (10×)	100 mL
Substrate Reagent	11 mL
Stop Solution	15 mL
Positive Control	150 µL
Negative Control	500 µL
Sealed Bag	1 piece
Plate Sealer	3 pieces
Product Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Additional Materials required

1. Microplate reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 25°C
4. Deionized or distilled water
5. Absorbent paper

4. Shipping and Storage

Store at 2-8℃. Avoid freeze. Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month. Expiration date is on the packing box.

5. Sample Preparation

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin-free. The serum must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8℃ for 1 week, or at - 20℃ for a long term storage.

Dilution Method

Please predict the concentration range of samples in advance and determine the dilution ratio through preliminary experiments or technical support recommendations.

6. Reagents Preparation

Wash Buffer: The 10×Concentrated Wash Buffer should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:9.

Antibody Working Mixture Solution: Mix in advance the Antibody Working Solution and the HRP Conjugate at a 1:1 volume ratio according to the number of samples N to be tested. (25 µl of Antibody Working Solution and 25 µl of HRP Conjugate per well). Prepare final volume for at least N+4 samples.

7. Assay procedure

1. **Bring all reagents to room temperature** (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

2. **Number**: number the samples and controls in order, and keep a record of control wells and sample wells. Take out the microtiter plate, set 2 well for the positive control and 2 wells for the negative control. Samples should be tested in duplicate.

3. **Add sample**: add 5 µL of positive/negative control to positive/negative control well. Add 5 µL of Serum to the sample well, and add 45 µL of Sample Diluent to the sample well.

4. **Antibody Working Mixture Solution**: Then add 50 µL of Antibody Working Solution and HRP Conjugate mixture to each well, gently oscillate to mix thoroughly. Cover the plate sealer and incubate at 25℃ for 90 min in shading light.

5. **Wash**: remove the liquid in each well. Immediately add 250 µL of Wash Buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).

6. **Color Development**: add 100 µL of Substrate Reagent into each well and mix thoroughly. Cover the plate sealer and incubate for 15 min at 25℃ in shading light.

7. **Stop reaction**: add 50 µL of Stop Solution into each well and mix thoroughly

8. **OD Measurement:** Measure the absorbance value (OD) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength). This step should be finished in 10 min after stop reaction.

8. Data analysis

Reference value

Normally, OD value of negative control > 0.6, the OD Average value of positive control / Average of negative control ODs < 0.3.

Interpretation of the results

1. PI=1 - (OD_{sample} - Average $OD_{negative\ control}$ / (Average $OD_{positive\ control}$ -Average $OD_{negative\ control}$) × 100%
2. Positive result: PI > 70%
3. Negative result: PI ≤ 70%
4. Unimmunized animal: positive result indicates that it may be infected with BC.
5. Immunized animal: The antibody levels at the time of the sample were monitored and recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

Limitations of this test method

1. This test is only used as the qualitative detection of Brucellosis antibody in the serum of swine, cattle, sheep and goat. A rough estimate (high, general, low) of the concentration of this antibody can be calculated according to the PI values.

2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

9. Important general notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA
7. The results shall depend on the readings of the Microplate Reader.
8. Each reagent is optimized for use in the AEES00743. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other AEES00743 with different lot numbers.
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolveyour problem, we will give you 100% of your money back.

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