Technical Manual

Cattle Brucellosis Antibodies ELISA Kit

- Catalogue Code: AEES00742
- Indirect ELISA Kit
- Research Use Only

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1. Description and Principle

The Assay Genie Cattle Brucellosis Antibodies ELISA Kit can assay for the specific antibodies in milk of cattle.

How Do Our ELISA Kit Assays Work?

This kit is based on Indirect-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with the Brucellosis (BC) antigen. During the reaction, the BC-Ab antibodies present in the sample will be bound to the antigen on the ELISA Microtiter plate. They will be detected by the horseradish peroxidase (HRP) conjugate. A substrate reagent is added for color development. The final yellow product is obtained after the reaction is ended by adding Stop Solution. The absorbance value of each well is measured using a microplate reader with 450 nm (630 nm) wavelength. There is a positive correlation between the OD value of samples and the concentration of BC- Ab

2. Key features and Sample Types

Sample type: milk

Sample type: cattle

ELISA Type: Indirect

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
Cattle Brucellosis Antibodies ELISA Kit	**96 assays**	**AEES00742**

Each kit contains reagents for 96 assays in a 96 well plate including:

Item	96T
Micro ELISA Plate	96 wells
Dilution Plate	96 wells
100X Concentrated HRP Conjugate	0,24 mL
Sample Diluent	80 mL
Concentrated Wash Buffer (25×)	50 mL
Substrate Reagent A	12 mL
Substrate Reagent B	12 mL
Stop Solution	12 mL
Positive Control	2 mL
Negative Control	2 mL
Sealed Bag	2 pieces
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 37°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

Milk: Take 2 mL of fresh sample into a 10 mL centrifuge tube, Centrifuge for 10 min at 4000 r/min. Avoid the upper layer of fat, take 50 µL of intermediate liquids (Solution 1) for another centrifuge tube.

Diluted milk: Dilute the Milk with the Diluent for step 1 at 1:1 (75 µL of Milk and 75 µL of Diluent, mix fully), mix fully.

6. Reagents Preparation

Wash Buffer: The 25×Concentrated Wash Buffer should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with distilled or deionized water at 1:24 (30 mL of 25×Concentrated Wash Buffer and 720 mL of deionized water, mix fully).

HRP Conjugate: The 100×Concentrated HRP Conjugate should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with Diluent at 1:99.

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 100 µL of Positive/Negative control to positive/negative control well, and add 100 µL of Diluted milk to the sample wells.

4. **Incubate**: cover the plate sealer and mix thoroughly, incubate at 37℃ for 30 min in shading light.

5. **Wash**: remove the liquid in each well. Immediately add 300 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. **HRP conjugate**: add 100 µL of HRP Conjugate into each well, cover the plate sealer and incubate at 37℃ for 30 min in shading light.

7. **Wash**: repeat step 5 for washing.

8. **Color Development**: Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B into each well and mix thoroughly. Cover the plate sealer and mix thoroughly, incubate at 37℃ for 10 min in shading light.

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

10. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm/630 nm wavelength. Note: Read the results within 5 min.

8. Data analysis

Reference value

Normally, the Average OD of negative control < 0.3 and Average OD of positive control ≥ 1.0.

Interpretation of the results

$$S/P = \frac{Average\ OD\ of\ sample\ control - Average\ OD\ of\ negative\ control}{verage\ OD\ of\ positive\ control - Average\ OD\ of\ negative\ control}$$

1. Positive result: S/P > 0.3.
2. Suspicious results: 0.2≤ S/P ≤0.3.
3. Negative result: S/P < 0.2.

Limitations of this test method

1. This test is only used as the qualitative detection of BC antibodies in milk of cattle. A rough estimate of antibody concentration (high, general, low) can be calculated based on the OD value.
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 AEES00742. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other AEES00742 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.

Contact Details

Email: info@assaygenie.com
Web: www.assayenie.com