Technical Manual

Brucellosis Antibodies ELISA Kit

- **Catalogue Code: ADES0029**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This kit is comprised by HRP conjugate, other reagents, ELISA Microtiter plate pre-coated with the Brucellosis (BC) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect BC antibody in serum of cattle, sheep, swine and goat. During the experiment, add control, samples and HRP Conjugate into the ELISA Microtiter plate. 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. There is a negative correlation between the OD value of samples and the concentration of BC-Ab. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether BC antibody exist in the sample.

2. Kit components

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

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

3. Experimental instrument

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor (10μl-100μl、100μl-1000μl）, EP tubes and disposable pipette tips
- 37° C incubator or water bath
- Deionized or distilled water
- Absorbent paper

4. 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 Microtiter plate should be covered by plate sealer. A void the kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. **Each reagent is optimized for use in the ADES0029. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0029 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.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum:** Use the conventional method to prepare serum, the serum must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8° C in 1 week, and it should be stored at - 20° C for a long term storage.
2. **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.
3. **Antibody Working Mixture Solution:** According to the number of samples N to be tested, the **Antibody Working Solution** and the **HRP Conjugate** were mixed in advance at a 1:1 volume ratio (on-demand mixing: the Antibody Working Solution and HRP Conjugate should be added 25μl*(N+4)*110%), respectively, and the Antibody Working Solution and the HRP Conjugate should be mixed evenly.

7. Assay procedure

Restore all reagents and samples to room temperature 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° C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Take out the microtiter plate, set 2 well for positive control and 2 wells for negative control. **Samples need test in duplicate.**
2. **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.
3. **Antibody Working Mixture Solution:** Then add 50 μL of **Antibody Working Mixture Solution** to each well, gently oscillate to mix thoroughly. Cover the plate sealer and incubate at 25° C for 90 min in shading light.
4. **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).
5. **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° C in shading light.
6. **Stop reaction:** add 50 μL of **Stop Solution** into each well and mix thoroughly
7. **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. Reference value

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

9. 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.

10. 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.

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Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com