Technical Manual

Goat and Sheep Brucellosis Antibodies ELISA Kit

- Catalogue Code: ADES0015
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant Brucellosis (BC) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect BC-Ab in serum of goat and sheep. During the experiment, add control and samples into the ELISA Microtiter plate, BC-Ab will be bound with the antigen on the ELISA Microtiter plate. Then wash the plate to remove unbound components, horseradish peroxidase (HRP) conjugate is added to each ELISA Microtiter plate well. The unbound HRP Conjugate will be removed by washing and substrate reagent is added for color development. At last, end the reaction by adding Stop Solution to produce a yellow product. There is a positive 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/96 wells *5
Dilution plate	96 wells/96 wells *5
HRP Conjugate	11 mL/26 mL*2
Sample Diluent	50 mL /200 mL
20×Concentrated Wash Buffer	40 mL /200 mL
Substrate Reagent A	6 mL /26 mL
Substrate Reagent B	6 mL /26 mL
Stop Solution	6 mL /26 mL
Positive Control	1 mL /2 mL
Negative Control	1 mL /2 mL
Plate Sealer	3 pieces/15 pieces
Sealed Bag	1 piece/5 pieces
Manual	1 copy

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

3. Other materials required but not supplied

- Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)
- High-precision transferpettor, 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 ADES0015. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0015 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.**Sample preparation**

1. 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. **Diluted serum**: Dilute the sample serum with the **Sample Diluent** at 1:9 (10 μL of sample serum and 90 μL of sample diluent, mix fully). The positive/negative control do not need to be diluted.
3. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

6. Assay procedure

Restore all reagents and samples 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° C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively. **Samples need test in duplicate.**
2. **Add sample:** add 100 μL of **positive/negative control** to positive/negative control well, and add 10 μL of **Diluted serum/plasma** and 90 μL of **Sample Diluent** to the sample wells.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37° C for 30 min in shading light.
4. **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).
5. **HRP conjugate:** add 100 μL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37° C for 30 min in shading light.
6. **Wash:** repeat Step 4 for washing.
7. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well. Cover the plate sealer and mix thoroughly, incubate at 37° C for 10 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) wavelength.

7. Reference value

Normally, the A-value of negative control ≤ 0.2 and the positive control ≥ 0.6.

8. Interpretation of the results

1. Positive result: OD ≥ 0.38
2. Negative result: OD < 0.38

9. Limitations of this test method

1. This kit is only used as the qualitative detection of Brucellosis antibody in serum of goat and sheep.

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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