Brucella Antibody (BRU Ab) ELISA Kit

Technical Manual

(ELISA)

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Product Information

Intended Use

Brucellosis, is a zoonotic systemic infectious disease caused by Brucella bacteria. Infected non-pregnant female animals typically show no apparent clinical symptoms, while the most significant symptom in pregnant female animals infected with Brucella is abortion.

This assay is designed to detect antibodies against BRU in the serum or plasma of bovine, sheep and goats. It can be used for the evaluation of the immunological efficacy of the BRU vaccine or as an adjunctive diagnostic tool for the disease.

Principle and Application

This kit comprises a coated Microtiter Plate with BRU antigen, antibody solution, HRP conjugate, and other accompanying reagents. It employs the principle of competitive enzyme-linked immunosorbent assay to detect antibodies against the BRU in the serum or plasma of bovine, sheep and goats. During the experiment, test sample, HRP conjugate and antibody solution are added to the plate. Following incubation, if BRU antibodies are present in the sample, they will compete with the antibody solution for binding to the antigens coated on the plate. The HRP conjugate will form a complex with the antibody solution and the coated antigens on the plate.

Subsequently, the plate is washed to remove any unbound components. Substrate reagents are then added to the wells. If BRU antibodies are present in the sample, they will inhibit the formation of a complex between the HRP conjugate, the antibody solution, and the coated antigens, thereby preventing any color development in the subsequent reaction. On the other hand, color development will occur if no BRU antibodies are present. The intensity of the color is inversely proportional to the concentration of specific antibodies in the sample.

Finally, a stop solution is added to terminate the reaction, turning the product yellow. The absorbance values in each well are measured at a wavelength of 450nm using a Microplate reader to determine the presence or absence of BRU antibodies in the sample.

Composition of the Kit

Reagent	Specification		
Microtiter Plate	96wells	96wells×2	96wells×5
Sample Diluent (yellow cap)	1×50mL	1×50mL	1×200mL
HRP conjugate (red cap)	1×11mL	2×11mL	2×26mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL	1×40mL	1×200mL
Substrate Reagent A (white cap)	1×6mL	1×11mL	1×26mL
Substrate Reagent B (black cap)	1×6mL	1×11mL (brown cap)	1×26mL
Stop Solution (yellow cap)	1×6mL	1×11mL	1×26mL
Positive Control (red cap)	1×1.0mL	1×1.5mL	1×2.0mL
Negative Control (green cap)	1×1.0mL	1×1.5mL	1×2.0mL
Adhesive Membrane	1	2	5
Sealed bag	1	1	2
Dilution plate	1	2	5
Instruction	1	1	1

Storage conditions

The kit shall be stored at 2-8 °C. Avoid moisture.

Shelf life: 12 months. Please use within 2 months after opening. The date of manufacture is presented in the label of the box.

Test Apparatus Required but Not Supplied

microplate reader, adjustable micropipette, constant temperature device (37°C), centrifuge.

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

1.Sample Preparation: The serum/plasma should be clear, without hemolysis or contamination. Samples can be stored at 2-8°C for up to 1 week, and for long-term storage, they should be kept at -20°C. It should be allowed to return to room temperature and mixed well before use.

2.Solution preparation: Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19). What obtained is the **working wash buffer.**

3.Negative and positive controls do not require dilution.

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

1.Put the required number of the wells on the plate and set up 2 wells each for negative/positive control.

2.Add 50μL of **negative control** to each negative control well. Then add 50μL of **positive control** to each positive control well. In each sample well, first add 40μL of the **working wash buffer**, then add 10μL of the serum sample. Next, add 50μL of the **HRP conjugate** and 50μL of **antibody solution** to all wells.

3.Shake the plate gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane and incubate at 37°C (water bath recommended) in the dark for 45 minutes.

4.Discard the liquid from the wells. Add 350μL of **working wash buffer** per well, let stand for 30 seconds, then discard. Repeat the washing process 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

5.First, add 50μL of **substrate reagent A** to each well, followed by 50μL of **substrate reagent B** per well. shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane, and incubate at 37°C in the dark for 15 minutes.

6.Add 50μL of **stop solution** to each well and shake gently by hand (or use a microplate shaker) for 5s. Read absorbance (**A value**) at 450nm with microplate reader (with 630nm as a reference wavelength). Finish this step within 10min.

Reference Value

Under normal experimental conditions, the A value of the negative control should be ≥1.0, and the A value of the positive control should be ≤ 50% of the A value of the negative control.

Interpretation of Test Results

1. $PI(\text{Percentage Inhibition}\%)=(1-\frac{A_S}{A_{NC}})\times 100\%$,

If PI is ≥50%, it is considered positive; if PI is <50%, it is considered negative.

A_S—the A value of the sample;

A_{NC}—the average A value of negative controls.

2. When the result is positive, it indicates the presence of brucellosis antibodies in animal. Further analysis should be conducted in conjunction with clinical and other methods.

Limitations of the Test Method

This test result is for screening purposes only and is not sufficient for confirmation.

Attention

1. During the experiment, gloves and lab coats should be worn. Strict and comprehensive disinfection and isolation protocols should be followed. All experimental waste should be treated as infectious material.

2. The stop solution is corrosive. Avoid contact with skin and clothing. If accidentally contacted, rinse immediately with a large amount of tap water.

3. When taking the microtiter plate out of a refrigerated environment, it should be brought to room temperature before opening the bag. Unused microplate wells should be stored in the sealed bag with a desiccant.

4. During washing, each well should be filled completely with liquid to prevent any residual enzyme on the well's rim from remaining unwashed.

5. The samples used for testing should be kept fresh.

6. The determination of test results must be based on the readings from the microplate reader.

7. Components from different lot numbers must not be mixed.