Cow Brucella Antibody (Anti-Brucella) ELISA Kit

Catalog No.: abx157258

Size: 96 tests

Detection Range: Qualitative

Sensitivity: Qualitative

Storage: Store all components at 2-8°C. Do not freeze.

Application: The qualitative detection of Cow Brucella Antibody in Cow milk.

Principle of the Assay: This kit is based on enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples and HRP-conjugated reagent are added to the wells and incubated. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient Brucella-Ab will produce a blue-colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow color is proportional to the Brucella-Ab amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of Brucella-Ab can be determined.

Kit components

1. 96-well microplate
2. 96-well dilution plate
3. Concentrated HRP Conjugate (100X): 0.24 ml
4. Sample Diluent: 80 ml
5. Concentrated Wash Buffer (25X): 50 ml
6. Substrate Reagent A: 12 ml
7. Substrate Reagent B: 12 ml
8. Stop Solution: 12 ml
9. Positive Control: 2 ml
10. Negative Control: 2 ml
11. Plate Sealer: 3
12. Hermetic Bag: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Distilled water
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator
8. Absorbent paper

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Milk**: Put 2 ml of sample into a tube and centrifuge for 10 mins at 4000 r/min. Avoiding the upper layer of fat, take 50 µl of sample and put into another tube. Dilute the sample at a 1:1 ratio (mix 75 µl of sample and 75 µl of Sample Diluent). Mix fully.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Serum samples should be clear, with no visible sediment or hemolysis.

2. Reagents

- **Wash Buffer**: Ensure the Concentrated Wash Buffer (25X) is bought to room temperature to ensure there is no sediment. Dilute the Concentrated Wash Buffer (25X) with distilled water at a ratio of 1:24 (mix 30 ml of Concentrated Wash Buffer (25X) with 720 ml of distilled water). Mix fully.
- **HRP Conjugate**: Ensure the Concentrated HRP Conjugate (100X) is bought to room temperature to ensure there is no sediment. Dilute the Concentrated HRP Conjugate (100X) with Sample Diluent at a ratio of 1:99. Mix fully.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Ensure all kit components and samples are bought to room temperature before use. Pre-heat the incubator and ensure it has reached a stable temperature before use. Pipette gently when plating samples and reagents to avoid foaming and bubbles.

1. Mark positions on the 96-well plate for each sample, the blank, and the controls. Set 2 wells for the negative control and positive control. *The sample wells must be tested in duplicate.*
2. Add 100 µl of Positive Control to the positive control well, and add 100 µl of Negative Control to the negative control well.
3. Add 100 µl of sample to the sample wells.
4. Mix thoroughly then cover with a plate sealer. Incubate at 37°C for 30 minutes in the dark.
5. After 30 minutes, remove the liquid in each well. Immediately add 300 µl of Wash Buffer to each well and wash. Repeat the wash procedure 5 times with 30 second intervals. Invert the plate on absorbent paper and pat to remove liquid. *If bubbles appear, use a clean pipette tip to prick them*.

6. Add 100 µl of HRP Conjugate into each well. Cover the plate with a plate sealer and incubate at 37°C for 30 minutes in the dark.
7. Remove the liquid in each well. Immediately add 300 µl of Wash Buffer to each well and wash. Repeat the wash procedure 5 times with 30 second intervals. Invert the plate on absorbent paper and pat to remove liquid. *If bubbles appear, use a clean pipette tip to prick them.*
8. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B into each well and mix thoroughly. Cover with a plate sealer and incubate the plate at 37°C for 10 minutes in the dark.
9. Remove the plate sealer and immediately add 50 µl of Stop Solution into each well. Mix fully.
10. Measure the absorbance value of each well using a microplate reader at 450 nm.

C. Calculation of Results

For each sample, calculate the Sample Value according to the following formula:

$$\text{Sample Value} = \frac{\text{Average OD of sample} - \text{Average OD of negative control}}{\text{Average OD of positive control - Average OD of negative control}}$$

Analysis

- **Positive Result:** Sample Value > 0.3
- **Negative Result:** Sample Value < 0.2

Note:

- Normally, the average OD of negative control is < 0.3, and the average OD of positive control is ≥ 1.0.
- This kit is qualitative and therefore a rough estimate of antibody concentration (low, general or high) can be determined based on the OD value.
- This kit is for research use only and the results are for reference only. It is recommended to use this kit in conjunction with another detection method.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.