Brucella Antibody (Anti-Brucella) ELISA Kit

Catalog No.: abx365011

Size: 96 tests

Detection Range: Qualitative

Sensitivity: Qualitative

Storage: Store all components at 4°C for up to 12 months. Use any opened components within 1 month. Avoid freezing.

Application: For qualitative detection of Brucella Antibody (Anti-Brucella) in serum samples.

Principle of the Assay

This kit is based on indirect enzyme-linked immuno-sorbent assay technology. The 96-well plate is pre-coated with the target antigen. Samples are added to the wells and incubated, then washed with wash buffer. Samples that contain anti-Brucella Antibody will bind to the pre-coated antigen to form an antigen-antibody complex. Unbound conjugates are washed away with wash buffer. Next, anti-Brucella primary antibody and HRP-conjugated detection antibody is added to the wells and incubated. TMB substrate is added, which is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The intensity of the yellow color is inversely proportional to the amount of sample anti-Brucella Antibody bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the presence of anti-Brucella antibody can be determined.

Kit components

1. 96-well microplate
2. Wash Buffer (10X): 100 ml
3. Sample Diluent: 100 ml
4. TMB Substrate: 11 ml
5. Stop Solution: 15 ml
6. Detection Reagent A: 1 vial
7. Detection Reagent B: 11 ml
8. Positive Control: 150 µl
9. Negative Control: 500 µl
10. Plate sealer: 3
11. Hermetic bag: 1

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

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.

- **Serum:** Prepare serum samples according to conventional methods. The tested serum should be clear, and free of any debris. Samples can be stored at 4°C for up to 1 week, or at -20°C for longer-term storage.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **1X Wash Buffer:** Dilute the concentrated 10X Wash Buffer with distilled water in a ratio of 1:9.
- **Detection Reagent A Working Solution:** Dissolve the lyophilized Detection Reagent A with 6 ml of dilute 1X Wash Buffer. Mix fully.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- At low temperatures, a crystalline precipitate may form in the 10X Wash Buffer. Before diluting, bring the 10X Wash Buffer to room temperature and swirl gently until the precipitate is fully dissolved.

B. Assay Procedure

1. Mark positions on the 96-well microplate for each sample and control. *Samples and the negative control should be tested in duplicate.*
2. Add 5 µl of positive control to the positive control well.
3. Add 5 µl of negative control to each negative control well.
4. Add 5 µl of sample to each sample well.
5. Add 45 µl of Sample Diluent to each sample well.
6. Add 50 µl of Detection Reagent A Working Solution to all wells.
7. Shake the plate gently to mix, then cover with a plate sealer and incubate at 25°C for 45 minutes.

8. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (250 µl) using a multi-channel pipette or auto-washer (a 30 second soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting.
9. Invert the plate and blot it against clean absorbent paper towels.
10. Add 100 µl of Detection Reagent B to each well.
11. Shake the plate to mix fully, then cover with a plate sealer and incubate at 25°C for 30 minutes.
12. Repeat Step 8, washing the plate 5 times with 1X Wash Buffer.
13. Add 100 µl of TMB Substrate to each well.
14. Shake the plate to mix fully, then cover with a plate sealer and incubate at 25°C for 10 minutes.
15. Add 50 µl of Stop Solution to each well and mix fully.
16. Measure the OD of each well with a microplate reader at 450 nm.

C. Analysis

1. Calculations:

Calculate the Percentage Inhibition (PI) using the following formula (using the mean OD values):

$$\mathrm{PI} = \frac{1 - \mathrm{OD}_{\mathrm{Sample}}}{\mathrm{OD}_{\mathrm{Negative\ Control}}} \times 100$$

Note:

- OD of the negative control should be > 0.6
- OD of the positive control should be < 0.3

2. Interpretation of results:

Samples:

If PI ≤ 70%, the test samples are considered negative.

If PI > 70%, the test samples are considered positive.

Positive result in unimmunized animals: This result indicates the animal may be infected with Brucella.

Positive result in recently immunized animals: This result indicates the animal may be responding to the vaccination.

These results are for reference only. It is strongly recommended to use a different, quantitative detection method to confirm and analyze the result.