Cow Brucella Antibody (Anti-Brucella) ELISA Kit

Catalog No: abx364922

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C.

Application: The qualitative detection of Anti-Brucella in Cow serum.

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 Anti-Brucella will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the Anti-Brucella amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of Anti-Brucella can be determined.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- 96-well Dilution Plate
- Wash Buffer: (20X) 15 ml
- Positive Control: 20 µl
- Negative Control: 20 µl
- Sample Diluent Buffer: 30 ml
- Detection Reagent: 75 µl
- Detection Reagent Diluent: 15 ml
- TMB Substrate A: 6 ml
- TMB Substrate B: 6 ml
- Stop Solution: 15 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C Incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Ice
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker
- Centrifuge

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles. Keep samples on ice during preparation. Equivalate samples to room temperature prior to analysis.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.

Notes:

- **Analyse samples at a 50-fold (1/50) dilution (e.g. add 5 µl of undiluted sample to 245 µl of Sample Diluent Buffer to obtain 250 µl of diluted sample).**
- Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Wash Buffer: Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Detection Reagent Working Solution Preparation:

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent 200-fold with Detection Reagent Diluent and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

Diluted Positive / Negative Control: Dilute Positive / Negative Control 50-fold with Sample Diluent Buffer (e.g. add 5 µl of undiluted Positive / Negative Control to 245 µl of Sample Diluent Buffer to obtain 250 µl of diluted Positive / Negative Control).

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure samples in duplicate.

1. Set 2 positive control, 2 negative control, and test sample wells on the pre-coated plate respectively, and record theirpositions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standrds and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Wash the plate once with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (0.5 mins 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. Invert the plate and blot it against clean absorbent paper towels.*

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3. Aliquot 100 µl of diluted negative control, 100 µl diluted positive control and 100 µl diluted serum into the set wells.
4. Cover the plate with a plate sealer and incubate for 30 mins at 37°C.
5. Remove the cover and discard the liquid. Wash the plate 3 times as directed in Step 2.
6. Aliquot 100 µl of Detection Reagent working solution to each well (except the blank well). Cover the plate with a plate sealer and incubate for 30 mins at 37°C.
7. Remove the cover, discard the liquid, and repeat the wash process as described above, 3 times.
8. Mix 50 µl of TMB Substrate A and 50 µl of TMB Substrate B. Add 100 µl of mixed TMB Substrate solution into each well. Cover the plate with the plate sealer and gently tap the plate to mix thoroughly. Incubate at 37°C for 15 mins. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
9. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

Data Analysis:

- Mean OD of the Positive Control should be ≥ 0.8
- Mean OD of the Negative Control should be ≤ 0.1
- (Mean $OD_{Negative\ Control}$ / Mean $OD_{Positive\ Control}$) x 100% should be < 10%

S/P = (OD_{sample} / $OD_{Positive\ Control}$) x 100%

If S/P ≥ 20%, the test samples are considered positive.

If S/P < 20%, the test samples are considered negative.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit:
https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.

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