Bovine Brucella antibody (Brucella-Ab) ELISA
Cat No: K04-S0054

Principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antigen specific to Brucella-Ab. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for Brucella is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain Brucella-Ab and HRP conjugated Brucella antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The presence of Brucella-Ab is determined by comparing with the CUTOFF value.

Intended Use:

This kit is used for the qualitative detection of Brucella--AB in Bovine Serum, Plasma, culture media or any biological fluid.

Materials provided:

1. Microtiter Coated Plate (96 wells)– 1 no
2. Positive Control– 0.5 ml
3. Negative Control– 0.5 ml
4. HRP Conjugate –6 ml
5. Wash Buffer (30X)-20 ml
6. Sample Diluent– 6 ml
7. Substrate A – 6ml
8. Substrate B– 6 ml
9. Stop Solution– 6 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 µl to 1000 µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Tubes to prepare standard/sample dilutions.
6. Timer.
7. Absorbent paper.
8. Incubator

Storage Information:

1. All reagents should be stored at 2 ºC to 8 ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25 ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. The kit cannot test samples which contain NaN_3, because NaN_3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
4. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make 1X Wash Solution, add 10 ml of 40X Wash Buffer in 390 ml of DI water.

Procedural Notes:

1. After taking the kit out from 2-8˚C environment, the kit should be stabilized for 30 minutes at room temperature and then used. If the coated strips haven't been used up after opening, then the remaining strips should be stored in a sealed bag.
2. For each step, add sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
3. The operation shall be carried out according to the instructions strictly. And test results must be based on the readings of the Microplate reader.
4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
5. All samples, washing buffer and each other component should be discarded according to infective material process.
6. Reagents not in used should be covered. Do not use reagent with different batches and use them before expiry date.
7. Substrate B is light-sensitive and so its prolonged exposure to light is forbidden.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that Samples should be run in duplicates.
2. Add **50ul negative control, positive control and sample (40 µl sample diluent and 10 µl test sample)** into respective well and in blank control well do not add any liquid .Shake the plate gently for 30 seconds to mix the reagents in the wells. *Care should be taken to avoid any spillage.*
3. Incubate at **37 °C for 30 minutes.**
4. Aspirate and wash plate 5 times with 300 µl **Wash Buffer (1x)** and blot residual buffer by firmly tapping plate upside down on an absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **50 µl** of **HRP Conjugate** to each well *(Do not add into blank control well)*
6. Incubate at **37 °C for 30 minutes**.
7. Aspirate and wash plate 5 times with 300 µl **Wash Buffer (1x)** and blot residual buffer by firmly tapping plate upside down on an absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
8. Add **50 µl** of **Substrate A** into all wells.
9. Add **50 µl** of **Substrate B** into all wells.
10. Incubate at **37 °C for 10 minutes.**
11. Add **50 µl of Stop Solution** into all wells*(Do not add into blank control well)*
12. Calibrate the plate reader with blank control well and read the plate using microwell plate reader at 450 nm

Interpretation of Results:

Colorimetric: Read O.D at 450nm with an ELISA reader.
Cut-off O.D=0.10+Average O.D of Negative Control.
O.D (If the O.D value of the Negative Control is lower than 0.05, calculate as per 0.05; if the O.D. value is more than 0.05, calculate as the actual data).

Positive: Sample O.D. ≥ 1.00 off O.D
Negative: Sample O.D. ≤ 0.15 off O.D

When average O.D of the Negative Control =<0.1 even average O.D of the Positive Control >=0.8 shows the kit and test is valid, otherwise, please repeat the test.
The critical value calculation: the critical value=the mean of the Negative comparative hole+0.15;
Negative determinant: if the sample OD value < the critical value, the Bovine (Brucella-Ab) is Negative;
Positive determinant: if the sample OD value ≥ the critical value, the Bovine (Brucella-Ab) is Positive

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.