Bovine Brucellosis Antibody ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT808001BZ5 (96 X 10 Tests)

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MANUAL VERSION 1.01

INTRODUCTION

The Brucellosis antibody ELISA kit is used to test Brucellosis antibody in bovine serum.

PRINCIPLE

This kit uses indirect ELISA method, Purified Bru antigen is pre-coated on enzyme micro-well strips. When testing, diluted serum sample is added and incubated in the well, if there is brucellosis virus specific antibody in the sample, it will combine with the pre-coated antigen. The uncombined antibody and other components are discarded with washing; and then enzyme conjugate is added. The uncombined enzyme conjugate is discarded by washing; TMB substrate is added in micro-wells, and the blue signal obtained by Enzyme catalysis is directly proportional to antibody content in sample. An ELISA reader at 450nm wavelength is used to measure the absorbance value in reaction wells after adding stop solution to stop the reaction.

MATERIALS SUPPLIED WITH THIS KIT

1	Brucellosis antigen coated microplate	96T×10	7	Stop solution	15ml×5
2	Enzyme conjugate	11ml×10	8	Washing solution 10X	100ml×5
3	Sample diluent	100ml×5	9	Serum dilution plate	2×5
4	Negative control	2ml×5	10	Adhesive Foil	4X5 pieces
5	Positive control	1.6ml×5	11	Instruction	1X5 pcs
6	Substrate	22ml×5			

MATERIALS REQUIRED BUT NOT PROVIDED

1)Microplate Reader (double-wave length: 450/630 nm).

2)Precise micropipette (single-channel 1-100µl, 0.5-10µl, multi-channel 30-300µl).

3)Constant temperature box or water bath.

4)Oscillator. 5)Microplate Washer.

6)Disposable tips (10µl, 200µl). 7)Deionized water.

SAMPLE PREPARATION

Take animal whole blood and separate serum by using regular centrifugation method. The serum should be bright with no hemolysis.

WASHING BUFFER PREPARATION

Keep 10X Concentrated washing buffer at room temperature before use, if there are salt crystals, shake to make them dissolved, then dilute buffer 10 times with distilled water or deionized water. The diluted washing buffer can be stored at 4℃ for about 1 week.

DILUTION OF SAMPLE

Dilute the sample to be tested (199ul sample dilution + 1ul sample serum) at 1:200 on the dilution plate. Note: negative and positive controls are not to be diluted. After taking each sample, replace the Micropipette tip and accurately record the position of each sample on the board. Each sample should be thoroughly mixed before adding to the Antigen coated microplate.

PROCEDURE

1) Take the antigen coated plate (the plate can be opened and used several times

according to sample quantity each time), add the diluted serum to reaction wells at 100µl per well; meanwhile, set 2 wells for positive control and 1 well for negative control. Positive control and negative control do not need to be diluted, take 100µl directly and add into the well, mix gently (do not overflow).

2) Cover it with adhesive plate sealer, incubate at 37 ℃ for 30 minutes.

3) Open the adhesive plate sealer, discard the liquid of the well, add diluted wash buffer to each well, 250µl per well, soak for 1min, then discard the liquid, repeat the above step for 5 times. At last wash step flip to dry with the absorbent paper.

4) Add Enzyme Conjugate, 100µl per well and cover it with Adhesive plate sealer. Incubate at 37 ℃ for 30 minutes;

5) Open the adhesive plate sealer, discard the liquid of the well, wash 5 times as earlier in Stem 3, and remember to flip plate in last wash to dry with the absorbent paper.

6) Add substrate: add Substrate 100µl per well and mix it evenly and cover it with adhesive plate sealer. Incubate at 37 ℃ in dark for 10 minutes;

7) Add stop solution 50µl/well to stop the reaction and measure the result in 10 minutes.

RESULTS

Read the OD value with ELISA Reader at 450nm (630nm as reference).

For the assay to be valid:

Negative control (N) OD value < 0.2,

Positive control (P) OD value ≥ 0.4;

Calculate method:

Sample OD value/Positive control OD average value= S/P value

Results interpretation:

S/P value ≥ 0.4 Positive

S/P value < 0.4 Negative

NOTE

1) Return all reagents into room temperature before use, shake it evenly before use, and store back to 2-8℃ after usage.

2) Do not mix use reagents from different kits and different lot numbers.

3) Substrate and stop solution can cause irritation to skin and eyes, therefore they should be handled carefully.

4) Do not expose Substrate to strong light and avoid contact with the oxidant.

5) Ag coated plates should be sealed and moisture-proof. Put back unused Microwell plate into dry foil bag and keep it ziplocked at 2-8 ℃. Once a plate seal is broke, use it completely within 30 days.

6) All wastes should be treated well to avoid pollution before discarding.

7) Strict compliance with the operating instructions will give the best and consistent results. Pipetting operation, timing, and washing processes must be precise.

8) Antigen coated plate is disposable, do not repeat use. The max volume of the plate is 300µl/well

Specifications: 96 wells × 10.

Expiry date: 12 months.

Storage: at 2-8 ℃ in dark. Do not freeze.