Brucellosis Antibody ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT808001BZ2

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MANUAL VERSION 1.01

INTRODUCTION

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

PRINCIPLE

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

MATERIASL SUPPLIED WITH THIS KIT

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

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, get serum by using regular method, the serum should be bright and 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 month.

PROCEDURE

1) Take the antigen coated plate (the plate can be open and used for several times according to sample quantity each time), add the diluted serum to reaction wells, 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 flap to dry with the absorbent paper;

4) Add Enzyme Conjugate, 100µl per well, Cover it with Adhesive plate sealer, incubate at 37 ℃ for 30 minutes;

5) Open the adhesive plate sealer, discard the liquid of the well, washing 5 times as earlier, and remember at last wash step to flap to dry with the absorbent paper;

6) Add substrate: add Substrate 100µl per well mix it, evenly then cover it with Adhesive plate sealer incubate at 37 ℃ in dark for 10 minutes;

7) Add stop solution 50µl/well to stop the reaction, 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 ≥ 0.3 Positive

S/P value < 0.3 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 no., prevent the reagents been polluted when using.

3) Substrate and stop solution may have irritation to skin and eyes, be careful to use.

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 sealed at 2-8 ℃.

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

7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process 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 × 4.

Expiry date: 12 months.

Storage: at 2-8 ℃, don't expose in strong light.

THIS MANUAL IS FOR REFERENCE PURPOSES ONLY.

ALWAYS REFER TO MANUAL SUPPLIED WITH KIT.