

Brucellosis antibody ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT917001BZ2



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INTRODUCTION

The Brucellosis antibody ELISA kit is used to test Brucellosis antibody in serum of bovine, sheep, pig and dog etc.

PRINCIPLE

This kit uses competitive ELISA method, Brucellosis antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample to the wells, after incubation, if there is Brucellosis specific antibody in the sample, it will combine with the pre-coated antigen. Discard the uncombined antibody and other components by washing; then add enzyme labeled anti-Brucellosis virus monoclonal antibody, antibody in sample block the combination of monoclonal antibody and pre-coated antigen; discard the uncombined enzyme conjugate with washing. Add TMB substrate in micro-wells, the blue signal by enzyme catalysis is in inverse proportion of antibody content in sample, use ELISA reader at 450nm wavelength to measure the absorbance A value in reaction wells after adding stop solution to stop the reaction.

MATERIALS SUPPLIED WITH THIS KIT

1	Brucellosis Ag coated microplate	96T×4	7	Stop solution	15×2 ml
2	Enzyme conjugate	20×2 ml	8	Washing solution 10X	100×2 ml
3	Sample diluent	100×2 ml	9	Dilution Plate	4
4	Negative control	200×2 µl	10	Adhesive Foil	8 pieces
5	Positive control	60×2 µl	11	Instruction	1 piece
6	Substrate	22×2 ml			

MANUAL VERSION 1.01

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°C for about 1 month.

PROCEDURE

- 1) Dilute the sample and controls (195µl sample diluent + 5µl sample serum or control) at 1:40 dilution on the dilution plate and mix by pipetting.
- 2) Take the pre-coated detection plate/strips (according to the sample number), add 20µl diluted serum sample to the test plate wells. At the same time set 2 wells for positive control and negative control. Add 20µl diluted control to the reaction well.
- 3) Then add enzyme conjugate 80µl per well with gentle shaking.
- 4) Cover the plate, incubate at 37 °C for 60 minutes.
- 5) Discard the liquid, add diluted washing buffer to each well, 250µl per well,

discard the liquid, repeat the above step for 5 times, at last washing flap to dry with the absorbent paper;

- 6) Add 100µl of substrate solution to each well, mix well, and close the plate to seal the plate at 37 °C for 15 minutes.
- 7) Add stop solution, 50µl/well to stop the reaction, measure the result within 10 minutes.

RESULTS

Set zero at blank control well, read the OD value at 450nm (with 620/630 nm as reference filter). For the assay to be valid:

OD value of NC > 0.5,

Positive control (P) blocking rate > 80%

Calculation method:

PI (blocking rate) = $\{1 - (\text{Sample OD} / \text{Negative control OD avg})\} \times 100\%$

Results interpretation

PI (blocking rate) > 70%: Positive

PI (blocking rate) ≤ 70%: Negative

NOTE

- 1) Return all reagents into room temperature before use, shake it evenly before use, and store back to 2-8°C 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) Brucellosis-Ag coated plates should be sealed and moisture-proof. Put back unused MicroWell plate into dry foil bag and sealed at 2-8 °C.
- 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) Brucellosis-Ag coated plates is disposable, do not repeat use.

Expiry date: 12 months.

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

**THIS IS A MANUAL FOR REFERENCE PURPOSES ONLY.
ALWAYS REFER TO MANUAL SUPPLIED WITH KIT.**