VERSION 1.01

BRUCELLOSIS ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Brucellosis Antibody ELISA Kit For Bovine, Goat,Sheep, Pig and Dog

Catalogue Number. IP100205

Introduction

The Brucellosis antibody ELISA kit is used to test Brucellosis antibody in serum of bovine, goat, sheep, pig and dog etc.This kit use competitive ELISA method, Brucellosis antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample, after incubation, if there is Brucellosis specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components with washing; then add enzyme labled 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 wavelenth to measure the absorbance A value in reaction wells after adding stop solution to stop the reaction

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	Brucellosis-Ag Coated plates 96wells	1/2 plates
2	Enzyme Conjugate	10/20 ml
3	10X Concentrated washing buffer	100 ml
4	Substrate	11/22 ml
5	Sample diluent	100 ml
6	Serum dilution plate	1/2 pieces
7	Stop solution	15 ml
8	Negative control	200 ul
9	Positive control	60 ul
10	Adhesive Foil	2/4 pieces
11	Instruction sheet	1 piece

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Washing buffer preparation

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4℃ for about 1 week.

Sample dilution

At serum dilution plate, dilute serum at 1:100 with sample diluent.
Notice: Negative control and Positive control do not need dilute. Exchange tip after taking sample every time, record the situation of the sample on plate accurately. Shake the sample evenly before adding it

Operation procedures

1) On serum dilution plate, dilute serum, negative control, positive control at 1:40 (for example: 195ul sample diluent + 5ul serum sample), mix it evenly;
2) Take Brucellosis-Ag Coated plates (open and take the quantity required for use according to sample quantity), add diluted serum into test wells, 20ul/well; meanwhile set 1 well for positive control and 2 wells for negative control, add diluted negative control and diluted positive control into its wells accordingly, 20ul/well;
3) Adding Enzyme Conjugate,80ul/well, shake gentle to mix it evenly, cover it with Adhesive Foil, incubate at 37 ℃ for 60 minutes;
4) Open the adhesive foil, discard the liquid of the well, add diluted washing buffer to each well, 250ul/well, discard the liquid, repeat the above step for 4-6 times, at last flap to dry with the absorbent paper;
5) Add substrate, 100ul/well, mix it evenly then cover it with Adhesive Foil, incubate at 37 ℃ in dark for 15 minutes;
6) Add stop solution, 50ul/well to stop the reaction, measure the result in 10 minutes.

Results judgement

Set zero at blank control well, read the OD value at 450nm (630nm as reference).
For the assay to be valid:
OD value of negative control(N) > 0.5, meanwhile positive value (P) blocking rate > 80%
Calculate method:
PI(blocking rate)= {1- (Sample OD value/ Negative control OD average value)}x 100%
Results interpretation
PI(blocking rate)> 70%: Positive
PI(blocking rate)≤ 70%: Negative

Notes

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. Brucellosis-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. Brucellosis-Ag coated plates is disposable, do not repeat use.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.