6 Q&A

Question	Answer	Solution
Weak signal	Solution dilution and Control formulation are incorrect	Samples and Controls are thoroughly dissolved and mixed with diluent, and used within the specified time.
	Insufficient dosage was added to the Microplate well	Check the function of the Micropipettes and calibrate if necessary. Make sure the tip is tightly attached to the Micropipettes.
	The response of antigen and	Check the reaction time to ensure that it is performed at the optimum temperature.
	Reagent expiration.	Check the validity period of the kit and do not use expired reagents.
	The label filter does not match.	Check the Microplate reader setup and label filter.
	Insufficient restored to room temperature of the kit.	Ensure that the kit is restored to room temperature (20-25℃) before use.
	Color development time is not enough.	Check whether the Substrate reaction time meets the requirements.
Background color dark	The color development time is too long and is not terminated in time.	Control the color development time and terminate the reaction in time.
	Nonspecific adsorption.	The reaction is strictly controlled at the most appropriate time and temperature.
	Insufficient Microplate washing.	Ensure adequate washing times.
		Ensure the soaking time of the washing liquid.
		Make sure to discard any residual solution before washing.
No signal	The Substrate Solution may be missed or the sequence of reagent addition is wrong.	Check test procedure, review the reagent addition sequence, repeat the test.
	Insufficient antibody dosage.	Check that the antibody dilution process is correct.
	The enzyme conjugate is contaminated.	Reformulate the reagent and repeat the test.
	The reagent or enzyme label Microplate is confused When different test kits are operated at the same time.	During the test, make labels to avoid confusion of reagents or enzyme label Microplate.
Gradient dilution showed single well abnormal results	The enzyme label Microplate are stacked.	Avoid overlapping of enzyme label Microplate.
	Inaccurate in gradient dilution.	Ensure the Micropipette is accurate and gradient dilution is performed correctly.
	The reagent evaporates during the reaction.	When incubating, seal with sealing plate film or add cover plate.
	The washing operation is uneven.	Make sure the washing process is operating properly.
	There is debris or water on the bottom of the enzyme label Microplate.	Clean the bottom of the plate when reading the result.
The standard curve is good but no signal in the sample well	Detectable substance concentration in the sample is low or not any.	A positive control was set up and the experiment was repeated.
	Sample matrix covering detection.	Select the appropriate dilution ratio, dilute the sample again and test again.
The standard curve is good but the sample signal is high	Detectable substance concentration in the sample exceeds the standard curve range.	Select the appropriate dilution ratio, dilute the sample again and test again.
Edge effect	Incubation temperature is not balanced.	Avoid incubation in areas with large temperature changes.

7 LIMITATION

Brucella Antibody ELISA Detection Kit is for *in vitro* veterinary diagnosis use only. All result should be considered with other clinical information available with veterinarian. It is suggested to apply a further confirmative method when positive result was observed.

VET Diagnostix

Brucella Antibody ELISA Detection Kit

EVEGEN, Hangzhou - Zhejiang - CHINA
www.vet-diagnostix.com Tel: +86-571-85366657
E-mail:sales@aijinbio.com Sales Tel: +86-571-85363586

Stored at 2 ~ 8℃.
Shelf life: 12 months



Version: 20240424A503E

1 INTENDED USE

Brucellosis is a zoonotic systemic infectious disease caused by brucellosis, which mainly affects animals, cattle, sheep, pigs, dogs, camels, deer and other animals. The test is used to detect antibodies against Brucella abortus (cattle) and Brucella Maltese (sheep and goats).
Specimen: serum

2 PRINCIPLE

The kit was pre-coated with Brucella lipopolysaccharide antigen in the micropores of the enzyme plate.In the experiment, the serum to be tested and the anti-Brucella lipopolysaccharide monoclonal antibody were added. After incubation, if the serum contains anti-Brucella antibody, it will compete with the anti-Brucella monoclonal antibody to bind the antigen and form an antigen-antibody complex. Wash to remove unbound antibodies and other components. Then the enzyme conjugate was added and combined with the antigen-antibody complex on the enzyme plate to form the antigen-antibody-enzyme conjugate complex. After washing, the unbound enzyme conjugate was removed, the color developing substrate solution was added into the microwell, and the blue signal generated by the enzyme catalysis was inversely proportional to the antibody content in the sample. After the reaction was terminated by adding the stop solution, the absorbance OD value in the reaction well was measured with the wavelength of 450nm using an 96-well microplate reader.

3 REAGENTS AND MATERIALS

Materials Provided

Component	Specification		
	96T	192T	480T
Enzyme Label Microplate	1pcs	2pcs	5pcs
Negative control	1ml×1	2ml×1	2.5ml×2
Positive control	1ml×1	2ml×1	2.5ml×2
Diluent 1	20ml×1	40ml×1	40ml×2
Enzyme Conjugate (50×)	1.2ml×1	2.4ml×1	3ml×2
Antibody Working Solution	8ml×1	16ml×1	40ml×1
20x Concentrated Wash Solution	25ml×1	50ml×1	50ml×2
Substrate Solution	12ml×1	22ml×1	26ml×2
Stop Solution	6ml×1	12ml×1	27ml×1
Sealing plate film	3pcs	6pcs	15pcs
Package insert	1pcs	1pcs	1pcs

Materials Required But Not Provided

Purified water
Pipettor
96-well Microplate reader (450nm)
Timer
Centrifuge (4000rpm)
Washing machine
Oven (37℃)

4 PRECAUTIONS

1. Do not use the test beyond its expiration date.
2. The substrate solution can be irritating to the skin.
3. The stop solution may be harmful if swallowed. It may cause sensitisation by skin contact (R22-43). Avoid contact with skin.
4. The components in this kit have been quality control tested as standard batch unit. Do not mix components from different lot numbers.
5. The reagents should be balanced to room temperature before the kit is used. Store at 2~8℃ after use.
6. The substrate solution can not be exposed to strong light or contact with oxidants.
7. The amount of serum to be tested is large, should first dilute all the serum to be tested, and then add to the antigen coated plate, so that the reaction time is consistent.
8. If the concentrated wash solution is found to have crystallization, it should be heated to dissolve it before use.
9. When pipetting, should be as accurate as possible to prevent bubbles.
10. The time and temperature specified in each operation step should be strictly observed.
11. All specimens are of potential infection. It must be strictly treated according to the rules and regulations by local states.
12. If the whole plate is not used at one time, it should be sealed and preserved in time.

5 TEST PROCEDURE AND RESULT INTERPRETATION

STEP 1 Test Procedure

1.1 Wash Solution Preparation

Working concentration washing liquid: Before use, the wash solution should be restored to room temperature (20-25℃) and shaken evenly to make it uniform. Then dilute 20 times the concentrated wash solution with purified water (for example, 5ml concentrated washing solution is added to 95ml purified water), mix well, and the diluted washing liquid can be stored at 2-8℃ for 7 days.

1.2 Sample Preparation

①Whole animal blood was taken, centrifuged at 4000rpm for 10 minutes, and supernatant was collected. Serum can also be coagulated naturally without hemolysis.
②When the sample size is large, in order to ensure the experimental reaction time is consistent. Mix the serum sample with the antibody working solution at 1:1 in the dilution plate (70μl serum sample + 70μl antibody working solution).The negative control and positive control are the same as the serum sample.

1.3 Test Procedure

①Take the enzyme label Microplate (according to the number of samples, can be disassembled and used several times), set up two wells each for negative and positive controls, add 100μl of the the premixed mixture to the enzyme label Microplate. Gently shake the sample in the well, using the sealing plate film to cover the plate, and incubate at 37℃ for 30 minutes.
②Open the sealing plate film, shake off the solution in the plate well, add 300μl of working concentration washing liquid to each well, and then discard the liquid in the well. Repeat the above steps 5 times, and finally pat dry on a clean absorbent paper.
③Diluted enzyme conjugate： Dilute the enzyme conjugate (50×) 50 times (for example: 20μl enzyme conjugate concentrate + 980μl diluent 1, the specific amount required is calculated according to the number of test samples), mix well and set aside.
④Add 100μl of Enzyme Conjugate to each well, cover the plate, and incubate at 37℃ for 30 minutes.
⑤Washing enzyme plate, the specific operation is the same as the above ②.
⑥ Add 100μl substrate solution to each well, cover the plate and incubate for 10 minutes at 37℃ away from light.
⑦ Add 50μl stop solution to each well, and determine the result within 5 minutes.

STEP 2 Result Interpretation

The OD450 value of each well was read on the 96-well microplate reader.

2.1 Validation

The average OD450nm value of positive control was < 0.35 and the average OD450nm value of negative control/positive control was ≥3.5, the experimental results were valid. Otherwise, the test should be repeated.

2.2 Calculation Method

For each sample, calculate the PI (Blockingr ate) as follows using the corrected sample and control values:

$$\mathbf{PI}\ (\text{Blocking rate}) = \left(1 - \frac{\mathbf{OD}_{\text{Sample}}}{\mathbf{OD}_{\text{NC}}}\right) \times 100\ \%$$

2.3 Result Interpretation

When PI < 20%, it is judged as negative.
When PI > 30%, it is judged as positive.
When 20% ≤ PI ≤ 30%, it is judged as suspicious, and suggest to repeat sampling and test one week later.