Brucellosis IgM ELISA KIT

Cat #: orb1085824 (manual)

Size: 96 tests

Product Usage

Detect the level of Brucella IgM antibody - Determine Wild Virus Infection.

Test method

ELISA-double antigen sandwich method

Storage conditions

2~8◦C refrigerated storage

Applicable sample

serum or plasma

Applicable species

sheep, cattle, pig, dog and human

Experimental Principle

According to the principle of double-antigen sandwich enzyme-linked immunosorbent assay, the testing kit firstly adds the sample to be tested into the microplate, the unknown antibody in the sample binds with the solid-phase antigen, forms a stable unknown antibody - antigen complex after washing, and then adds the conjugated antigen to bind with the antigen complex to form a conjugated antigen - antibody - antigen complex. After washing, add the chromogenic agent and stop the reaction, and measure the absorbance OD value.

Product Composition

Reagent name	Specifications	Quantity	Storage Conditions
Antigen-Coated Slats	8×12	1 plate	4℃
Enzyme Conjugated Antigen	10 mL	1 bottle	4℃
Critical Value Control	0.5 mL	1 bottle	4℃
Negative Control	0.5 mL	1 bottle	4℃
Positive Control	0.5 mL	1 bottle	4℃
Chromogenic reagent A	6 mL	1 bottle	4℃

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Chromogenic reagent B	6 mL	1 bottle	4℃
Stop Solution	6 mL	1 bottle	4℃
Concentrated wash solution	50 mL	1 bottle, 1:50 diluted	4℃

Precautions

1. This reagent is used for in vitro testing of reagent, and cannot be mixed with different total batch numbers. Remove each reagent from the box before use and allow to stand at room temperature for at least 30 min.
2. Dilute the concentrated wash solution with medical distilled water for 1:50 times, and incubate the concentrated wash solution for 15 min at 37℃ after crystallization. During the rinsing of the plates, be sure to clean and dry the residual liquid in the wells. After the test sample is added, pay attention to shake the microwell reaction strip slightly so that the liquid in the well is well mixed.
3. During large-batch specimen operation, pay attention to control the dispensing time and try to avoid slow dispensing speed or too long dispensing interval.
4. Adjust the pipette scale and set the volume value. The new tip should be first aspirated and drained back into the original container and the pipette tip used for disposable.

Operation Procedure

1. Add test sample: Add 100 microliters of the negative and positive control articles and the specimen to be tested respectively into the corresponding microwells. The stopper of the control article should be capped on the original corresponding bottle mouth. Do not mix.
2. Incubation: Seal the dispensed microplate with a seal, and vibrate the microplate with a microoscillator or by hand for 30 seconds. The sealed plates were incubated in water bath 37℃ for 24 h.
3. Washing: After incubation, remove the seal and discard the liquid in the microwell, add 220 ~ 300 microliters of diluted washing solution, and wash the microwell plate for 3~5 times. After washing, dry the residual liquid in the wall with absorbent paper, and wipe the liquid and fingerprint on the outside of the plate.
4. Enzyme Conjugated Antigen: Add 100 microliters of Enzyme Conjugated Antigen to the wells.
5. Incubation: Seal the dispensed microplate with a seal, and vibrate the microplate with a microoscillator or by hand for 30 seconds. The sealed plates were incubated in water bath 37℃ for two hours.
6. Washing: After incubation, remove the seal and discard the liquid in the microwell, add 220 ~ 300 microliters of diluted washing solution, and wash the microwell plate for 3~5 times. After washing, dry the residual liquid in the wall with absorbent paper, and wipe the liquid and fingerprint on the outside of the plate.
7. Color development: Place the washed microplate on the workbench, add 50μL of Chromogenic reagent A for each well, and then add 50μL of Chromogenic reagent B (or add 100 μL/well after mixing according to the ratio of 1：1 in advance). After mixing, place it in a light-proof environment, and allow it to stand at room temperature 18~25◦C for 20 min.

8. Termination: Remove the plate from the light-protected environment, add 50 microliters of Stop Solution per well, and gently mix.
9. Measurement: Place the microplate of the terminated reaction in the reading slot of the microplate reader, use the detection wavelength of 450 nm to measure and read the absorbance value of each well (or use the dual wavelengths of 450 nm and 630nm), and the data reading should be completed within 30 min.

Result judgment

1. IgM antibody Negative: Sample OD value less than cut-off value, healthy.
2. Positive IgM antibody: Sample OD value ≧ Critical Value, infection.
3. IgM antibody positive: Sample OD value (90days after vaccination) ≧Critical Value, infection.

Quality Control

1. Sensitivity: 100%
2. Specificity: No cross-reaction with other substances in the sample.
3. Safety: Coated-antigen, conjugated antibody, controls are immunogenic only, non-microbial toxicity, and transmissibility.
4. Within-batch difference: Coefficient of variation CV<10% (n=3).
5. Inter-batch difference: Coefficient of variation CV<15% (n=9).
6. Test conditions: OD value of positive control was ≧ 1.0.

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