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Bovine Ehrlichiosis ELISA Kit

96 Tests

Catalogue Number: SL0082Bo

Store all reagents at 2-8℃

Validity Perid: six months

For samples:

In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READTHROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Bovine Ehrlichiosis ELISA Kit

FOR RESEARCH USE ONLY

Drug Names

Generic Name: **Bovine Ehrlichiosis ELISA Kit.**

Purpose

This kit allows for the determination of Ehrlichiosis in Bovine serum, tissue, cell culture supernates and other biological fluids.

Principle of the assay

The kit assay Ehrlichiosis level in the sample, use Purified Ehrlichiosis antibody to coat microtiter plate wells, make solid-phase antibody, then add Ehrlichiosis to wells, Combined With Ehrlichiosis, after washing and removing non-combinative antigen and other components ,then Combined Ehrlichiosis antibody which with HRP labeled become antibody –antigen–enzymeantibody complex, after washing Completely, Add TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. Compared with the CUTOFF value, according to this to judge Ehrlichiosis exist in the sample or not.

Materials provided with the kit

Materials provided with the kit	96 determinations	Storage
User manual	1	R.T.
Closure plate membrane	2	R.T.
Sealed bags	1	R.T.
Microelisa stripplate	1	2-8℃
Negative control	0.5ml×1 bottle	2-8℃
Positive control	0.5ml×1 bottle	2-8℃
HRP-Conjugate reagent	6ml×1 bottle	2-8℃
Sample diluent	6ml×1 bottle	2-8℃
Chromogen Solution A	6ml×1 bottle	2-8℃
Chromogen Solution B	6ml×1 bottle	2-8℃
Stop Solution	6ml×1 bottle	2-8℃
Wash Solution	（20ml×30 fold）×1bottle	2-8℃

Specimen requirements

1. **Serum-** coagulation at room temperature 10-20mins，centrifugation 20-min at the speed of 2000-3000r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
2. **Plasma-**use suited EDTA or citrate plasma as an anticoagulant, mix 10-20mins, centrifugation 20-min at the speed of 2000-3000r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
3. **Urine**-collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.
4. **Cell culture supernatant**-detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000r.p.m. remove supernatant, detect the composition of cells, Dilute cell suspension with PBS （PH7.2-7.4）, Cell concentration reached 1 million/ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

5. **Tissue samples**- After cutting samples, check the weight, add PBS（PH7.2-7.4）, Rapidly frozen with liquid nitrogen, maintain samples at 2-8℃ after melting, add PBS（PH7.4）, Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.
6. Extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can’t, specimen can be kept in -20 ℃ to preserve, Avoid repeated freeze-thaw cycles.
7. Can’t detect the sample which contain NaN3, because NaN3 inhibits HRP active.

Assay procedure

1. Number: to sample correspond microtitration well and Number Sequence, each plate should be set feminine comparison 2 wells, masculine comparison 2 wells, blank comparison 1 well(don’t add sample and HRP-Conjugate reagent to blank comparison well, other each step the operation are same).

2.Add sample： separately add Positive control and Negative control 50μl to the Positive and Negative well . add Sample dilution 40μl to testing sample well, then add testing sample 10μl. add sample to the bottom of ELISA plates coated well , don’t touch the well wall as far as possible, and Gently mix.

3.Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at 37℃.

4.Configurate liquid: 30-fold wash solution diluted 30-fold with distilled water and reserve.

5.Washing： Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

6.Add enzyme：Add HRP-Conjugate reagent 50μl to each well, except blank well.

7.Incubate：Operation with 3.

8.Washing：Operation with 5.

9.Color：Add Chromogen Solution A 50μl and Chromogen Solution B 50μl to each well, evade the light preservation for 15 min at 37℃

10.Stop the reaction：Add Stop Solution 50μl to each well, Stop the reaction(the blue color change to yellow color).

11.Assay ：take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.

Important notes

1. The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature, ELISA plates coated if has not use up after opened, the plate should be stored in Sealed bag.
2. Washing buffer will Crystallization separation, it can be heated the water helps dissolve when dilute. Washing does not affect the result.
3. Add Sample with sampler Each step, And proofread its accuracy frequently, avoids the experimental error. Add sample within 5mins, if the number of sample is much, recommend to use Volley.
4. If the testing material content is excessively higher (The sample OD is bigger than the first standard well), please dilute Sample (n-fold). Please dilute and multiplied by the dilution factor（×n×5）.
5. Closure plate membrane only limits the disposable use, to avoid cross-contamination.
6. The substrate evade the light preservation**.**
7. Please according to use instruction strictly. The test result determination must take the microtiter plate reader as a standard.
8. All samples, washing buffer and each kind of reject should according to infective material process.
9. Do not mix reagents with those from other lots.

Determine the result

Test validity: the average of Positive control well≥1.00; the average of Negative control well ≤0.10.

Calculate Critical(CUT OFF) : Critical= the average of Negative control well +

0.15.

Negative control: sample OD< Calculate Critical(CUT OFF) is Ehrlichiosis Negative control.

Positive control: ample OD≥ Calculate Critical(CUT OFF) is Ehrlichiosis Positive control.

Storage and validity

1. Storage: 2-8℃.
2. Validity: six months.