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Bovine Viral Diarrhea Virus (BVDV) ELISA Kit(Qualitative)

Product Number: ELK063

Shipping and Storage

1. Reagent kit storage: 2-8℃.
2. Validity period: 6 months.

Component

Component	96T
30× concentrated wash buffer	20mL×1
Enzyme conjugate	6mL×1
Microplate (Coated)	12 wells×8 strips
Sample diluent	6mL×1
Chromogen substrate A	6mL×1
Chromogen substrate B	6mL×1
Stop solution	6mL×1
Positive control	0.5mL×1
Negative control	0.5mL×1

Description

This kit uses a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect bovine viral diarrhea virus (BVDV) in specimens. Using purified bovine viral diarrhea virus (BVDV) antibodies coated on a microplate, solid-phase antibodies can be prepared that can bind to the viral diarrhea virus (BVDV) in the sample. After washing to remove unbound antigens and other components, they can then bind to HRP labeled viral diarrhea virus (BVDV) antibodies to form antibody antigen enzyme-linked antibody complexes. After thorough washing, TMB substrate is added for color development. TMB is converted to blue under the catalysis of HRP enzyme and to the final yellow under the action of acid. Measure the absorbance (OD value) at a wavelength of 450nm using an enzyme-linked immunosorbent assay (ELISA) reader, and compare it with the CUTOFF value to determine the presence or absence of bovine viral diarrhea virus (BVDV) in the specimen.

Application

This kit is used to detect the level of viral diarrhea virus (BVDV) in bovine serum, plasma, and related liquid samples.

Specimen requirements

1. Serum: Blood naturally coagulates at room temperature for 10-20 minutes, centrifuge for about 20 minutes (2000-3000 revolutions per minute). Carefully collect the supernatant. If precipitation occurs during storage, centrifuge again.
2. Plasma: EDTA or sodium citrate should be selected as anticoagulants according to the requirements of the specimen. After mixing for 10-20 minutes, centrifuge for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. If sediment forms during storage, it should be centrifuged again.
3. Urine: Collect with a sterile tube and centrifuge for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. If sediment forms during storage, centrifuge again. Refer to the implementation for pleural effusion, ascites, and cerebrospinal fluid.
4. Cell culture supernatant: When detecting secreted components, collect them using sterile tubes. Centrifuge for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. When detecting intracellular components, dilute the cell suspension with PBS (pH 7.2-7.4) to a cell concentration of around 1 million/ml. By repeatedly freezing and thawing, cells are destroyed and intracellular components are released. Centrifuge for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. If

precipitation forms during storage, it should be centrifuged again.

5. Tissue specimen: After cutting the specimen, weigh it. Add a certain amount of PBS, pH 7.4. Quickly freeze and store in liquid nitrogen for future use. The specimen remains at a temperature of 2-8℃ even after melting. Add a certain amount of PBS (pH 7.4) and homogenize the specimen thoroughly by hand or using a homogenizer. Centrifuge for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. After packaging, one portion is to be tested, and the rest is to be frozen for future use.
6. Extract the specimen as soon as possible after collection, according to relevant literature, and conduct experiments as soon as possible after extraction. If the experiment cannot be conducted immediately, the specimen can be stored at -20℃, but repeated freezing and thawing should be avoided.
7. Samples containing NaN3 cannot be detected because NaN3 inhibits the activity of horseradish peroxidase (HRP).

Protocol

1. Number: Number the corresponding micropores of the sample in sequence. Each plate should have 2 negative control wells, 2 positive control wells, and 1 blank control well (blank control wells do not contain samples or enzyme-linked immunosorbent assay reagents, and all other steps are the same).
2. Sample addition: Add 50μL of negative control and positive control to the negative and positive control wells respectively. Then add 40μL of sample diluent to the test sample well, followed by 10μL of the test sample. Add the sample to the bottom of the enzyme-linked immunosorbent assay plate well, avoiding touching the well wall as much as possible. Gently shake and mix well,
3. Incubation: Cover the plate with a sealing film and incubate at 37℃ for 30 minutes.
4. Preparation: Add distilled water to 600ml of 30× concentrated washing solution and set aside for later use.
5. Washing: Carefully remove the sealing film, discard the liquid, shake dry, fill each hole with detergent, let it stand for 30 seconds, then discard. Repeat this process 5 times and pat dry.
6. Enzyme addition: Add 50μL of enzyme labeled reagent to each well, except for blank wells.
7. Incubation: Follow the same procedure as in 3.
8. Washing: The operation is the same as 5.
9. Color development: Add 50μL of color developing agent A50 to each well, then add 50μL of color developing agent B50, gently shake and mix, and develop color at 37℃ in the dark for 15 minutes
10. Termination: Add 50μL of termination solution to each well to terminate the reaction (at this point, the blue color turns yellow).
11. Measurement: Zero the blank well and measure the absorbance (OD value) of each well in sequence at a wavelength of 450nm. The measurement should be conducted within 15 minutes after adding the termination solution.

Calculation and result judgment

Experimental effectiveness: The average value of positive control wells is ≥ 1.00; Negative control mean ≤ 0.20;

CUT OFF calculation: CUT=mean value of negative control wells+0.15;

Negative determination: Samples with OD values less than the critical value (CUT OFF) are negative for bovine viral diarrhea virus (BVDV);

Positive determination: Samples with OD value ≥ critical value (CUT OFF) are positive for bovine viral diarrhea virus (BVDV).

Note

1. Strictly follow the instructions for operation, and components from different batches of this reagent must not be mixed.
2. The kit should be balanced at room temperature for 15-30 minutes before being taken out of the cold storage environment. If the enzyme coated plate is not used up after opening, the Flat noodles should be stored in a sealed bag.
3. Concentrated washing solution may precipitate crystals. When diluting, it can be dissolved by heating in a water bath, and washing does not affect the results.
4. The sealing film is only for one-time use to avoid cross contamination.
5. Please store the substrate away from light.

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6. The determination of experimental results must be based on the reading of the enzyme-linked immunosorbent assay (ELISA) reader. When using dual wavelength detection, the reference wavelength is 630nm
7. All samples, detergents, and various waste materials should be treated as infectious agents. The termination solution is 2M sulfuric acid, and safety must be taken into account when using it.