Precautions

Kit components should be handled and disposed of as potentially hazardous. Do not eat, drink, or smoke where serum samples and kit reagents are handled. Do not pipette by mouth. Some reagents may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents, or reagents from other kits or serials. Do not mix reagents from different serials of this same product.

Component B, Positive Control, contains sodium azide as a preservative.

Component C, Negative Control, contains sodium azide as a preservative.

Component D, Antibody-Peroxidase Conjugate, contains ProClin 300, methylisothiazolone and bromonitrodioxane as preservatives.

Component E, 50X Wash Solution Concentrate, contains benzalkonium chloride as a preservative.

Component G, Stop Solution, contains sodium fluoride.

USDA Veterinary License No. 332 Version 130920

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vmrd Veterinary Medical Research & Development

FOR VETERINARY USE ONLY

USDA Product Code 5010.20

BLUETONGUE VIRUS ANTIBODY TEST KIT, cELISA

Assay instructions for catalog numbers: 287-2 and 287-5

General Description

This competitive, enzyme-linked, immunosorbent assay (cELISA) detects bluetongue virus antibodies in ruminant sera. Sample serum bluetongue virus antibody inhibits binding of horseradish peroxidase (HRP)-labeled bluetongue-virus-specific monoclonal antibody to bluetongue viral antigen coated on the plastic wells. Binding of the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent color product development. Strong color development indicates little or no blockage of HRP-labeled monoclonal antibody binding and therefore the absence of bluetongue virus antibody in sample sera. Weak color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of bluetongue virus antibodies in sample sera.

Kit Contents

Component	287-2	287-5
A Antigen-Coated Plates	2 plates	5 plates
B Positive Control	4 ml	4 ml
C Negative Control	4 ml	4 ml
D Antibody-Peroxidase Conjugate	16 ml	16 ml
E 50X Wash Solution Concentrate	60 ml	60 ml
F Substrate Solution	30 ml	30 ml
G Stop Solution	30 ml	30 ml
This insert		

Materials Required But Not Included in the Test Kit

Single and multichannel adjustable-volume pipettors and disposable plastic tips, test tubes or non-antigen-coated transfer plate(s), ELISA microplate absorbance spectrophotometer with 620, 630 or 650 nm filter, deionized or distilled water, paper towels, graduated cylinder, timer, multichannel pipettor reservoirs, wash bottle, manual multichannel washing device or automatic plate washer

Storage and Stability

Store all reagents at 2-7°C. **Do not freeze.** Unopened reagents will remain stable until the expiration date when stored as instructed. **Do not use test kit past the expiration date printed on the box.**

Preparation

a. **Warm reagents:** Bring the serum samples, reagents and plate(s) to room temperature (23 ± 2°C) prior to starting the test.

b. **Prepare controls and samples:** Positive and Negative Controls are provided ready to use. Load in duplicate, regardless of the number of serum samples to be tested. When whole plates are used, it is best to put the controls in wells on different areas of the plate. Controls must be loaded on every plate.

   Serum samples are tested UNDILUTED.

c. **Prepare plates:** Remove the plate(s) from the foil pouch(es) (A). If applicable: Return any unused strips to the pouch and securely seal it. Extra pouches and sealer are available from VMRD. Place strips to be used in the frame and number the top of each strip to maintain orientation. Always mark the strips in case they dislodge from the frame during washing.

d. **Prepare conjugate:** Antibody-Peroxidase Conjugate (D) is READY-TO-USE. No dilution is necessary.

e. **Prepare wash solution:** Prepare 1X Wash Solution by diluting 1 part of the 50X Wash Solution Concentrate (E) with 49 parts of deionized or distilled water. Approximately 1 ml is needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc.

Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 25 µl, transfer controls and serum samples into the Antigen-Coated Plate (A). Serum samples and controls should be loaded into the Antigen-Coated Plate (A) as quickly as possible. When running more than two strips, we recommend that the serum samples and controls be first loaded into a transfer plate and then transferred to the Antigen-Coated Plate (A) using multi-channel pipetting equipment. The sample volume in the transfer plate must be in excess of 25 µl in order to transfer 25 µl from it. Firmly tap each of the long sides of the loaded assay plate 7 times for a total of 14 taps to make sure the samples coat the bottom of the wells. Use care not to spill samples from well to well. Incubate the plate 15 minutes at room temperature (23 ± 2°C).
2. **Add conjugate:** After the 15-minute incubation, add 25 µl of Antibody-Peroxidase Conjugate (D) to each well. Mix the plate contents by firmly tapping each of the long sides of the plate 7 times for a total of 14 taps. Incubate for 15 minutes at room temperature (23 ± 2°C).
3. **Wash wells:** After the second 15-minute incubation, wash the plate 3 times: *If an automatic washer is used*, place the plate on the washing apparatus and wash plate 3 times, filling the wells each time with 1X Wash Solution. *If manual washing is used*, dump well contents and remove remaining sera and controls by sharply striking the inverted plate 4 times on a clean paper towel, striking a clean area each time. Immediately fill each well with 1X Wash Solution using a multichannel filling device or a wash bottle. Empty the wash solution from the plate and strike the inverted plate sharply on a clean paper towel as above. Fill and empty the plate by the same method 2 additional times for a total of 3 washes.
4. **Add substrate solution:** Add 50 µl of Substrate Solution (F) to each well. Firmly tap each of the long sides of the loaded assay plate 7 times for a total of 14 taps to make sure the substrate coats the bottom of the wells. Incubate 10 minutes at room temperature (23 ± 2°C). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
5. **Add stop solution:** Add 50 µl of Stop Solution (G) to each well. Mix the well contents by firmly tapping each of the long sides of the plate 7 times for a total of 14 taps. *Do not empty wells.*
6. **Read and record the test results:** Immediately after adding the Stop Solution, the plate should be read on a microplate absorbance spectrophotometer. Set the optical density (O.D.) reading wavelength to 620, 630 or 650 nm. Blank the instrument on air and read plate(s). Some readers require an empty well on the plate for blanking. In this case, no reagents should be added to this well.
7. Return all remaining kit reagents to 2-7°C for storage.

Test Validation

- The mean of the **Negative Control** optical densities must be >0.300 and <2.000.
- The mean of the **Positive Control** optical densities must be < 50% (one-half) of the mean of the **Negative Control** optical densities.

Interpreting the Results

- Test samples are positive if they produce an optical density < 50% of the mean of the **Negative Controls**.
- Test samples are negative if they produce an optical density ≥ 50% of the mean of the **Negative Controls**.

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