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 0.09% sodium azide as a preservative.

Component C, Negative Control, contains 0.09% sodium azide as a preservative.

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

Component F, Stop Solution, contains sodium flouride.

Version 180327

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Veterinary Medical Research & Development

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VLN/PCN 332/5010.20

BLUETONGUE VIRUS ANTIBODY TEST KIT, cELISA v2

Assay instructions for catalog number: 5010.20-2

General Description

This competitive, enzyme-linked, immunosorbent assay (cELISA) detects antibodies to bluetongue virus (BTV) in ruminant sera. Sample serum BTV antibody inhibits binding of horseradish peroxidase (HRP)-labeled BTV-specific monoclonal antibody to BTV antigen coated on the plastic wells of the microtiter plate. 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 BTV 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 BTV antibodies in sample sera.

Kit Contents

Component	
A Antigen-Coated Plates	2 plates
B Positive Control	4 ml
C Negative Control	4 ml
D Antibody-Peroxidase Conjugate	16 ml
E Substrate Solution	30 ml
F Stop Solution	30 ml
Instructions for Use	

Materials Required But Not Included in the Test Kit

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

Storage and Stability

Store all reagents at 2-8°C. **Do not freeze.** Reagents will remain stable until the expiration date when stored as instructed. **Do not use test kit past the expiration date.**

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:** The Positive and Negative Controls (B & C) and the test serum samples must be diluted 1/2 in Antibody Peroxidase Conjugate (D) for use in this test. These dilutions should be made in a non-antigen-coated transfer plate. First, add Antibody Peroxidase Conjugate (D) to the appropriate wells of the transfer plate according to the setup record. Add an equal volume of Positive Control (B), Negative Control (C), or sample to each well, and cycle the pipettor at least three times to mix. Change tips between samples. The volume in the transfer plate must be in excess of 50 µl in order to transfer 50 µl from it. Load the Positive Control (B) in duplicate and the Negative Control (C) in triplicate, 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 plates. The controls must be loaded every time the assay is performed and on each plate if multiple plates are used.

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. 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.

Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50 µl, load diluted 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. Change tips between rows or columns. Tap the side of the Antigen-Coated Plate (A) several times to make sure the samples coat the bottom of the wells. Use care not to spill samples from well to well. Incubate the plate 30 minutes at room temperature (23 ± 2°C).

2. **Wash wells:** After the 30-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 distilled or deionized water.

   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 distilled or deionized water 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.

3. **Add substrate solution:** Add 50 µl of Substrate Solution (E) to each well. Tap the side of the loaded assay plate several times 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.*

4. **Add stop solution:** Add 50 µl of Stop Solution (F) to each well. Tap the side of the loaded assay plate several times to mix the Substrate Solution and the Stop Solution. *Do not empty wells.*

5. **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 (OD) reading wavelength to 620, 630 or 650 nm 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.

6. Return all remaining kit reagents to 2-8°C for storage.

Calculation of % Inhibition (% I):

% I = 100 [1-(Sample OD ÷ NC OD)]

Test Validation

- The mean of the **Negative Controls (NC)** must produce an optical density > 0.300 and < 2.000.
- The mean of the **Positive Controls** must have an inhibition of ≥ 60%.

Interpreting the Results

- If a test sample produces ≥ 60% inhibition, it is positive.
- If a test sample produces < 60% inhibition, it is negative.

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