

SETUP RECORD

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	A	B	C	D	E	F	G	H

Catalog Nos.: 287-2 and 287-5

Kit Serial: _____

Case ID: _____

Date: _____



**BLUETONGUE VIRUS ANTIBODY TEST
KIT, cELISA**

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

USDA Product Code 5010.20 • For veterinary use only

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 with Setup Record for recording sample identifications and results.

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 reader or spectrophotometer with 620, 630 or 650 nm filter. Deionized or distilled water. Paper towels. Multichannel pipettor reservoirs. Graduated cylinder. Wash bottle, manual multichannel washing device or automatic plate washer. Timer.

Storage and Stability

Store all reagents at 2-7°C (35-45°F). **Do not freeze.** 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.**

Precautions

Do not eat, drink or smoke where serum samples and kit reagents are handled. Do not pipette by mouth. Some reagents contain sodium azide or sodium fluoride. These chemicals may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents, or reagents from other kits. Do not mix reagents from different lots of this same product.

Preparation

- a. **Warm up reagents:** Bring the serum samples, reagents and plate(s) to room temperature prior to starting the test.
- b. **Position controls and samples:** Run Positive and Negative Controls 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 parts of the plate. Controls must be run on every plate. Enter the control and serum sample IDs on a photocopy of the attached Setup Record.
- 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. Call VMRD for extra pouches and sealer. Place strips to be used in the frame and number the top of each strip to maintain orientation with the Setup Record. Always mark the strips in case they fall out of 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 one 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.
- f. **Prepare serum samples:** Serum samples are tested UNDILUTED.

Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 25 µl, transfer controls and serum samples to the Antigen-Coated Plate (A) according to the Setup Record. **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 (21-25°C; 70-77°F), uncovered.
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 an additional 15 minutes at room temperature (21-25°C; 70-77°F), uncovered.
3. **Wash wells:** After the second 15-minute incubation, wash the plate three times:
If an automatic washer is used, place the plate on the washing apparatus and wash plate three times, filling the wells each time with 1X Wash Solution.
If manual washing is used, dump contents of the wells into a sink and remove the remaining sera and controls by sharply striking the inverted plate four times on a clean paper towel, striking a clean area each time. Immediately fill each well with 1X Wash Solution using repeating syringe with a manifold, wash bottle or multichannel pipettor. Dump out the Wash Solution and strike the inverted plate sharply on a clean paper towel as above. Repeat the washing procedure two more times (three washes total).

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 (21-25°C; 70-77°F), uncovered. 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 result:** Immediately after adding the Stop Solution, the plate should be read on a plate reader. Set the optical density (O.D.) reading wavelength to 620, 630 or 650 nm. Blank reader 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 (35-45°F) for storage.

Test Validation

- The mean of the **Negative Controls** must produce an optical density greater than 0.300 and less than 2.000.
- The mean of the **Positive Controls** must produce an optical density less than or equal to 50% (one-half) of the mean of the **Negative Controls**.

Interpreting the Results

- Test sera are positive if they produce an optical density less than 50% of the mean of the **Negative Controls**.
- Test sera that produce an optical density greater than or equal to 50% of the mean of the **Negative Controls** are negative.

USDA Veterinary License No. 332

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