



# BOVINE LEUKEMIA VIRUS ANTIBODY TEST KIT

Assay instructions for catalog numbers: 284 and 284-5

USDA Product Code 5505.20 • For veterinary use only

## General Description

This enzyme-linked immunosorbent assay (ELISA) detects antibodies to Bovine Leukemia Virus (BLV) glycoprotein (gp51) in bovine sera. Sample serum antibodies bind to BLV gp51 molecules attached to the plastic wells of the microtiter plate. Binding of these serum antibodies is detected by reaction with horseradish peroxidase (HRP)-labeled affinity-purified goat antibodies to bovine immunoglobulins. Attached HRP-labeled antibodies are detected by addition of enzyme substrate and quantitated by subsequent blue color product development. Strong color development indicates the presence of antibody to BLV gp51 in the sample serum. Very weak or no color development indicates the absence of antibody to BLV gp51 in the sample serum.

## Kit Contents

<b>Component</b>	<b>284</b>	<b>284-5</b>
<b>A</b> Antigen-Coated Plates	1 plate	5 plates
<b>B</b> Positive Control	3.6 ml	3.6 ml
<b>C</b> Negative Control	3.6 ml	3.6 ml
<b>D</b> 100X Antibody-Peroxidase Conjugate	150 µl	500 µl
<b>E</b> Conjugate Diluting Buffer	14 ml	60 ml
<b>F</b> 10X Wash Solution Concentrate	120 ml	2 x 120 ml
<b>G</b> Serum Diluting Buffer	30 ml	2 x 120 ml
<b>H</b> Substrate Solution	20 ml	60 ml
<b>I</b> Stop Solution	20 ml	60 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 cylinders. 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 thimerosal, sodium azide or sodium fluoride. These chemicals may be harmful if ingested. If ingested, seek medical attention. Material Safety Data Sheet (MSDS) is available on request. 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 samples, reagents and plate(s) to room temperature prior to starting the test.
- b. **Position controls and samples:** Run the Positive Control (**B**) in triplicate and the Negative Control (**C**) 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:** Prepare fresh 1X Antibody-Peroxidase Conjugate by diluting one part of the 100X Antibody-Peroxidase Conjugate (**D**) with 99 parts of Conjugate Diluting Buffer (**E**). Example: For 96 wells, mix 60  $\mu$ l of 100X Antibody-Peroxidase Conjugate (**D**) with 5.940 ml of Conjugate Diluting Buffer (**E**) to yield 6 ml of ready-to-use (1X) Antibody-Peroxidase Conjugate. Fifty microliters (50  $\mu$ l) are needed per well.
- e. **Prepare wash solution:** Prepare fresh 1X Wash Solution by diluting one part of the 10X Wash Solution Concentrate (**F**) with nine parts of deionized or distilled water. Example: For 96 wells, mix 20 ml 10X Wash Solution Concentrate (**F**) with 180 ml deionized or distilled water to yield 200 ml of ready-to-use 1X Wash Solution. (You will need 1.5 ml total per well of ready-to-use 1X Wash Solution.) Allow extra for reservoirs, washing equipment, tubing, etc. Extra diluted Wash Solution may be kept in a tightly-sealed container at room temperature for later use.
- f. **Prepare serum samples:** Dilute serum samples 1:25 with Serum Diluting Buffer (**G**) in test tubes or in a clean non-antigen-coated transfer plate. At least 65  $\mu$ l of each diluted sample are required per well because of transfer loss. Mix tubes by vortexing and mix transfer plate wells by micropipettor action. If using a transfer plate, add 65  $\mu$ l per well of the Positive Control (**B**) and the Negative Control (**C**) to the transfer plate, as indicated on the Setup Record. **DO NOT DILUTE THESE CONTROL SERA.**

## Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50  $\mu$ l, transfer controls and serum samples to the Antigen-Coated Plate (**A**) according to the Setup Record. Tap the side of the loaded assay plate 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 20 minutes at room temperature (21-25°C, 70-77°F).
2. **Wash wells:** After the 20-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).

3. **Add conjugate:** Add 50  $\mu$ l of 1X Antibody-Peroxidase Conjugate to each well. Tap the side of the loaded assay plate several times to make sure the conjugate coats the bottom of the wells. Incubate the plate 20 minutes at room temperature (21-25°C, 70-77°F).
4. **Wash wells:** After the 20-minute incubation, wash the plate three times as described in step 2.
5. **Add substrate solution:** Add 50  $\mu$ l of Substrate Solution (**H**) to each well. Tap the side of the loaded assay plate several times to make sure that the substrate coats the bottom of the wells. Incubate 20 minutes at room temperature (21-25°C; 70-77°F). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
6. **Add stop solution:** Add 50  $\mu$ l of Stop Solution (**I**) to each well. Gently mix the well contents by tapping the side of the plate several times. *Do not empty wells.*
7. **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.
8. Return all remaining kit reagents to 2-7°C (35-45°F) for storage.

## Test Validation

- The **Positive Control** must produce a mean optical density greater than or equal to 0.250 and less than 2.000 (i.e.,  $0.250 \leq \text{O.D.} < 2.000$ ).
- The **Negative Control** must produce a mean optical density less than 0.200 (i.e.,  $\text{O.D.} < 0.200$ ).

## Interpreting the Results

- Test sera are positive for BLV antibody if they produce an optical density greater than or equal to the mean of the **Positive Control** O.D.s.
- Test sera are negative for BLV antibody if they produce an optical density less than the mean of the **Positive Control** O.D.s.

## U.S. Veterinary License No. 332

Version 060801



# SETUP RECORD

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Date: \_\_\_\_\_ Case ID: \_\_\_\_\_ Kit Serial: \_\_\_\_\_ Catalog Nos.: 284, 284-5