

NOTE: Although the published specificity (website and product catalog) on our BLV test kit is 100% based on a specific sample set, no diagnostic test kit is always 100% specific on all sample populations. Since market introduction of our BLV kit, occasional false positives have been encountered. We advise all users that when BLV prevalence is low, positive samples should be confirmed by some other method, particularly where valuable animals may be involved and/or when BLV status is used as the SINGLE criterion for disposition of animals. Whenever import restrictions do not prohibit it, VMRD will provide reference assay service for positives of high-value animals or for positives in low-prevalence situations. We are not capable of testing large numbers of samples, and therefore cannot provide this reference assay service for all positives found.

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, 100X Antibody-Peroxidase Conjugate, contains thimerosal as a preservative.

Component E, Conjugate Diluting Buffer, contains thimerosal as a preservative.

Component G, Serum Diluting Buffer, contains sodium azide as a preservative.

Component I, Stop Solution, contains sodium fluoride.

USDA Veterinary License No. 332

Version 120806

BOVINE LEUKEMIA VIRUS ANTIBODY TEST KIT

Assay instructions for catalog numbers: 284 and 284-5

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

Component	284	284-5
A Antigen-Coated Plates	1 plates	5 plates
B Positive Control	3.6 ml	3.6 ml
C Negative Control	3.6 ml	3.6 ml
D 100X Antibody-Peroxidase Conjugate	0.15 ml	0.5 ml
E Conjugate Diluting Buffer	14 ml	60 ml
F 10X Wash Solution Concentrate	120 ml	2 × 120 ml
G Serum Diluting Buffer	30 ml	2 × 120 ml
H Substrate Solution	20 ml	60 ml
I Stop Solution	20 ml	60 ml
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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, 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.** 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 samples, reagents and plate(s) to room temperature ($23 \pm 2^\circ\text{C}$) prior to starting the test.
- b. **Prepare controls and samples:** Test serum samples must be diluted 1/25 with Serum Diluting Buffer (G) for use in the test. It is recommended that these dilutions be made in a non-antigen-coated transfer plate or in test tubes. At least 65 µl of each diluted sample are required per well because of transfer loss. Mix transfer plate wells by micropipettor action and mix tubes by vortexing. Positive and Negative Controls are provided ready to use. **DO NOT DILUTE THESE CONTROL SERA.** Load Positive Control (B) in triplicate and 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 areas of the plate. Controls must be run on every plate.
- 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:** Prepare 1X Antibody-Peroxidase Conjugate by diluting 1 part of the 100X Antibody-Peroxidase Conjugate (D) with 99 parts of Conjugate Diluting Buffer (E). Example: For 96 wells, mix 60 µl of 100X Antibody-Peroxidase Conjugate (D) with 5.940 ml of Conjugate Diluting Buffer (E) to yield 6 ml of 1X Antibody-Peroxidase Conjugate. Fifty microliters (50 µl) are needed per well.
- e. **Prepare wash solution:** Prepare 1X Wash Solution by diluting 1 part of the 10X Wash Solution Concentrate (F) with 9 parts of deionized or distilled water. Approximately 1.5 ml are needed per well. Allow extra for reservoirs, washing equipment, tubing, etc.

Test Procedure

1. **Load controls and serum samples:** Using a pipettor set at 50 µl, load 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 50 µl in order to transfer 50 µl from it. 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 ($23 \pm 2^\circ\text{C}$).

2. **Wash wells:** After the 20-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 contents of the wells into a sink and remove the 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 pipettor, wash bottle or a syringe with a manifold. Dump out the Wash Solution and strike the inverted plate sharply on a clean paper towel as above. Repeat the washing procedure 2 more times (3 washes total).
3. **Add conjugate:** Add 50 µl of diluted (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 for 20 minutes at room temperature ($23 \pm 2^\circ\text{C}$).
4. **Wash wells:** After the 20-minute incubation, wash the plate 3 times as in Step 2.
5. **Add substrate solution:** Add 50 µl 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 ($23 \pm 2^\circ\text{C}$). Avoid leaving the plate in direct sunlight. *Do not empty wells.*
6. **Add stop solution:** Add 50 µl of Stop Solution (I) 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.*
7. **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.
8. Return all remaining kit reagents to 2-7°C for storage.

Test Validation

- The **Positive Controls** must produce a mean O.D. ≥ 0.250 and < 2.000 .
- The **Negative Control** must produce a mean O.D. < 0.200 .

Interpreting the Results

- Test samples 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 samples are negative for BLV antibody if they produce an optical density less than the mean of the **Positive Control** O.D.s.