VDPro® Bovine Leukosis Ab b-ELISA

CAT. NO. EB-BLV-01

GENERAL DESCRIPTION

Bovine leukemia virus (BLV) is a retrovirus that may cause lymphosarcoma in cattle. The virus resides in blood lymphocytes where circulating antibodies are unable to neutralize it. Therefore, once an animal is infected with BLV, it is infected for life.

BLV is economically significant to the producer because of premature culling or death as a result of lymphosarcoma. Another concern is the condemnation of carcasses at slaughter, which has a significant economic impact on the dairy and cattle industries. Losses from export restrictions are another economic concern of BLV infection. Countries that have bovine leukosis control programs require BLV-free certification prior to shipping cattle to their regions. Moreover, exporters of semen are under increasing pressure to ensure that their product is from a BLV-free animal in a BLV-free herd.

VDPro® Bovine Leukosis Ab b-ELISA is designed to detect presence of anti-gp51 BLV antibody in bovine serum. The specimen is added to the BLV Antigen Coated Plate followed by the incubation of the mAb (HRPO Anti-BLV Conjugate). If the BLV gp51 specific antibodies are present in the specimen, the chromogenic signal by substrate will be disappeared.
The result is calculated by using percentage of the sample to negative control ratio (percentage of S/N ratio).

KIT COMPONENTS

Reagents	192 tests	480 tests
① BLV Antigen Coated Plate	2 plates	5 plates
② 10X Washing Buffer	120㎖X1	240㎖X1
③ Dilution Buffer	30㎖X1	60㎖X1
④ HRPO Anti-BLV Conjugate	40㎖X1	70㎖X1
⑤ Positive Control, PC	1.0㎖X1	2.0㎖X1
⑥ Negative Control, NC	1.0㎖X1	2.0㎖X1
⑦ TMB Substrate	30㎖X1	70㎖X1
⑧ Stop Solution	20㎖X1	40㎖X1
⑨ Sealing Film	2 sheets	5 sheets
⑩ Instruction Manual	1 copy	1 copy

MATERIALS

1) Micropipette and tip
2) Dilution Plate for dilution of serum
3) 8 or 12 channels micropipette
4) Distilled water
5) Reader : ELISA reader (450 nm)
6) Automated or semi-automated washer for ELISA plate

PREPARATION

1. All reagents must be allowed to come to room temperature (20~25℃) before use. Mix reagents by gentle swirling.
2. 1X washing buffer preparation
   1) Shake 10X Washing Buffer(②) gently.
   2) Dilute 1 part of 10X Washing Buffer(②) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~25℃).
3. Sample preparation
   1) Use the serum samples as fresh as possible
   2) Use fresh samples for the best result. Serum samples can be stored at 2~8℃ for less than 3 days or –20℃ for a longer period. Do not freeze and thaw serum samples repeatedly. **Sera with hemolysis or bacterial contamination are not suitable for the analysis!**
   3) Visible solid materials in serum samples should be separated by centrifugation.
4. Samples, Positive control and Negative control dilution
   1) Prepare dilution plate (96-well, not offered) or suitable tubes.
   2) Dilute 60㎕ of test serum, Positive (PC, ⑤) and Negative (NC, ⑥) Control with 60㎕ of Dilution Buffer (③) in a plate well or a suitable tube.
   3) Diluted Samples and PC, NC should be mixed prior to dispensing into the Antigen Coated Plate(①).
5. TMB Substrate (⑦) should be warmed up for 30 minutes at room temperature (20~25℃) before use (10㎖/plate). If stored at low temperature, the color development may be poor.

TEST PROCEDURE

1. **Remove the BLV Antigen Coated Plate (①) from protective foil pouch.**
2. Add 100㎕ of the diluted serum sample to each well of the plate, keeping the wells A1 and A2 for Positive Control (PC, ⑤) and the wells B1 and B2 for Negative Controls (NC, ⑥). **Use care not to spill samples from well to well.**
3. Add 100㎕ of diluted Positive Controls (PC, ⑤) and Negative (NC, ⑥) Controls in the designated wells.
4. Cover the plate with enclosed Sealing Film (⑨).
5. Incubate the plate for one hour (±2 min) at 37℃.
6. Wash each well 3 times with 1X washing buffer (300 ㎕ per well). And remove contents of well at each washing steps. After final washing, hit plate to the paper towel briefly to remove solution.
7. Add 100㎕ of HRPO Anti-BLV Conjugate (ready for use, ④) to each well.
8. Cover the plate with enclosed Sealing Film (⑨).
9. Incubate the plate for one hour (±2 min) at 37℃.
10. Wash the plate as described in Step 6.
11. Add 100㎕ of TMB Substrate (⑦) to each well.
12. Cover the plate with enclosed Sealing Film (⑨).

13. Incubate the plate for 10 minutes (±1 min) at room temperature (20~25℃). Protect the plate from direct light exposure.
14. Add 50㎕ of Stop Solution (⑧) to each well of the plate. Shake the test plate shortly (5~10 sec.). **Be careful not to spill.**
15. Measure and record the A (450nm) for samples and controls immediately.
16. Validate and calculate the results.

- ◆ If the color reaction of the negative control is not visible even after 10 minutes from the naked eye, the substrate reaction time can be extended to 20 minutes.
- ◆ For precise measurement, it is recommended to remove any obstructions such as bubbles in the plate well before reading.

Plate template example (1-well Test)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	13	21	29	37	45	53	61	69	77	85
B	NC	NC	14	22	30	38	46	54	62	70	78	86
C	1	7	15	23	31	39	47	55	63	71	79	87
D	2	8	16	24	32	40	48	56	64	72	80	88
E	3	9	17	25	33	41	49	57	65	73	81	89
F	4	10	18	26	34	42	50	58	66	74	82	90
G	5	11	19	27	35	43	51	59	67	75	83	91
H	6	12	20	28	36	44	52	60	68	76	84	92

RESULT INTERPRETATION

1. Validate if the mean OD of the PC is lower than 0.300 and the mean OD of the NC is higher than 0.500. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate a percentage of S/N ratio by dividing the mean OD value of a sample by the mean OD value of the NC as below.

$$\text{S/N(\%)} = \frac{\text{Sample OD}}{\text{Mean of NC OD}} \times 100$$

3. Result interpretation
   1) Test samples having < 40 S/N(%) are positive.
   2) Test samples having ≥ 40 S/N(%) are negative.

S/N(%) value	Interpretation
S/N(%) < 40	Positive
S/N(%) ≥ 40	Negative

4. Example of result calculation and interpretation
   1) ODs of PC : 0.150, 0.157
      Mean OD = (0.150 + 0.157) / 2 = 0.154 (valid)
   2) ODs of NC :1.129, 1.130
      Mean OD = (1.129 + 1.130) / 2 = 1.130 (valid)
   3) OD of Sample : 0.387
      S/N(%) value of the sample = (0.387 / 1.130) X100 = 34.2
   4) Result interpretation: Positive

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~25℃) before use. Mix reagents by gentle swirling. After use, return to 2~8℃.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use of the product.
3. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
4. Do not use expired or contaminated reagents.
5. Do not use reagents from other kits or lots.
6. Do not mix reagents from different lots of this same product.
7. Do not expose the reagents to excessive heat or direct light during storage and incubation.
8. Incomplete washing adversely may affect the result and precision of the assay.
9. Avoid microbial contamination of the reagents.
10. Avoid contamination of the TMB Substrate(⑦) with the HRPO Anti-BLV Conjugate(④).
11. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
13. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
14. Use care not to spill samples from well to well.
15. Deionized water or equal must be used to prepare the washing buffer.
16. Unused strips should be stored in the sealed foil pouch at 2~8℃. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

Store all reagents at 2~8℃. Do not freeze. Reagents remain stable until the expiration date when stored as instructed.

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