Avian Leukosis Complex Antigen ELISA Kit

Catalog No.: abx365015

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: For qualitative detection of Avian Leukosis Complex Antigen in Chicken Cloacal Secretions and Egg White.

Introduction: Avian sarcoma leukosis virus (ASLV) is an endogenous retrovirus that infects and can lead to cancer in chickens; experimentally it can infect other species of birds and mammals. ASLV replicates in chicken embryo fibroblasts, the cells that contribute to the formation of connective tissues. Different forms of the disease exist, including lymphoblastic, erythroblastic, and osteopetrotic. Avian sarcoma leukosis virus is characterized by a wide range of tumors, the most common of which are lymphomas. Lymphoid leukosis is the most common form of this disease and with typical presentation of gradual onset, persistent low mortality, and neoplasia of the bursa. The disease is also characterized by an enlarged liver due to infiltration of cancerous lymphoid cells. In addition, other abdominal organs and the bursa of Fabricius are often infected.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. A 96 well plate has been pre-coated with an antibody specific to Avian Leukosis Complex Antigen. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagent is added to the wells. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the Avian Leukosis Complex Antigen amount bound on the plate. The O.D. absorbance is measured spectrophoto-metrically at 450 nm in a microplate reader, and the presence of Avian Leukosis Complex Antigen can be determined.

Kit components

1. One pre-coated 96-well microplate (8 × 12 well strips)
2. Positive Control: 2 ml
3. Negative Control: 2 ml
4. Wash buffer (25X): 25 ml. Dilution: 1:25
5. HRP Conjugate Reagent (RTU): 25 ml
6. Stop solution: 15 ml
7. TMB substrate A: 15 ml
8. TMB substrate B: 15 ml
9. Plate sealer: 3
10. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes
7. Deionized or distilled water
8. 0.01 M PBS (pH 7.4)
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Cloacal Secretions:** A swab should be inserted into the cloacal cavity and rotated repeatedly on the inner wall. Insert the swab immediately into a tube of 1 mL x 0.01 M PBS (pH 7.4). Stir the swab until the sample is dissolved into the diluent fully. Analyze the sample immediately or aliquot and store at -20°C.
- **Egg White:** Collect Egg White and assay immediately or aliquot and store at -20°C.

Note:

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 25 ml of concentrated wash buffer into 600 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use.

1. Set positive/negative controls, test sample and control (zero/blank) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 100 µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 100 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
4. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 min away from light.
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (260 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 100 µl of HRP conjugate reagent into each well (except the blank well). Add the solution at the bottom of each well without touching the side wall.
7. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 min away from light.
8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 5.
9. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 10 min. Avoid exposure to light.
10. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.

11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

1. **Calculations:**
   Mean absorbance of the positive control should be ≥ 1.
   Mean absorbance of the negative control should be ≤ 0.3.

   R = (Sample OD - Average negative control OD) / (Average positive control OD - Average negative control OD)

2. **Interpretation of results:**
   If the postive control value is ≥ 1 and the negative control value is ≤ 0.3, the test is valid, otherwise, the test is invalid.
   Samples:
   If R < 0.2, the test samples are considered negative.
   If R ≥ 0.2, the test samples are considered positive.
   Precision:
   Variable coefficient (CV%) ≤ 15%

D. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
4. It is recommended measuring each controls and samples in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells do not let the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled..
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. The TMB Substrate B is easily contaminated; protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate the TMB substrate at room temperature prior to use. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.