Duck Virus Hepatitis (DVH) ELISA Kit

Catalog No.: abx054976

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: For qualitative detection of DVH in Duck Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: Duck Hepatitis is caused by the enteroviruses DHV-1 and DHV-3. It is a fatal disease of ducklings causing opisthotonus and hepatitis. DHV-1 is found worldwide and causes disease in young ducklings, usually <6 weeks of age and spreads rapidly within a flock. It is the most virulent of the DHV species.

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 DVH. 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 DVH amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and the presence of DVH can be determined.

Kit components

1. One pre-coated 96-well microplate (8 × 12 well strips)
2. Positive Control: 0.5 ml
3. Negative Control: 0.5 ml
4. Wash buffer (30X): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP Conjugate Reagent (RTU): 6 ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. 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 to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
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 at 1:5 dilution (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.

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

» Store samples undiluted. Once ready to analyze, thaw samples and dilute 1:5.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set positive/negative, test sample and control (zero) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 50 µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Samples should be diluted 1:5. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
4. Seal the plate with a cover and incubate at 37°C for 30 min.
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) 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 50 µl of HRP conjugate reagent into each well (except control well). Add the solution at the bottom of each well without touching the side wall.
7. Seal the plate with a cover and incubate at 37°C for 30 min.
8. Remove the cover and repeat the aspiration/wash process 5 times.

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 15 min. Avoid exposure to light.
10. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
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 absobance of the positive control should be ≥ 1.00.
   Mean absobance of the negative control should be ≤ 0.10.
   CUT OFF value = Negative control + 0.15.
2. **Interpretation of results:**
   If the postive control value is ≥ 1.00, and negative control value is ≤ 0.10, the test is valid, otherwise, the test is invalid.
   Samples:
   If O.D. of samples < CUT OFF, the test samples are considered negative.
   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

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. Wash buffer may crystallize and separate. If this happens, please heat the tube to dissolve.
4. It is recommended measuring each controls and samples in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
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. Do not use expired components or components from a different kit.
10. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. TMB Substrate solution should also be protected from light. 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.