Chicken Newcastle Disease virus Antibody (NDV-Ab) ELISA

Cat No: K16-S0099

Ver 1.0

Principle:
This is enzyme-linked immunosorbent assay (ELISA) to assay the level of Chicken Newcastle Disease virus Antibody (NDV-Ab) in samples. Addition of controls or sample to microtiter well which is pre-coated with Anti-NDV, if Newcastle Disease virus Antibody (NDV-Ab) antibody present, it will bind to the capture antibody coated on plate during incubation. After washing HRP conjugate is pipetted to form immune complex. Unbound HRP conjugate will get removed by washing step after incubation. Then addition of TMB Substrate develops blue color during incubation period and reaction will get stop after addition of stop solution with development of yellow color. The concentration of the Chicken Newcastle Disease virus Antibody (NDV-Ab) of sample is directly proportional to the yellow color developed in well and will be positively correlated.

Intended Use:
This kit is used for the qualitative detection of Chicken Newcastle Disease virus Antibody (NDV-Ab) in Chicken serum, plasma and other biological samples. The Kit is For Laboratory / Research Use Only.

Materials provided:
1. Microtiter Coated Plate (96 wells) – 1 no
2. Positive Control – 0.5 ml
3. Negative Control – 0.5 ml
4. HRP Conjugate – 6 ml
5. Wash Buffer (20X) - 25 ml
6. Sample Diluent – 6 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:
1. 37℃ incubator
2. Standard microplate reader.
3. Precision pipettes and Disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution
6. Absorbent paper.

Storage Information:
1. All reagents should be stored at 2ºC to 8ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

Note: Samples to be used within 5 days may be stored at 2-8ºC, otherwise samples must be stored at -20ºC (≤1 month) or -80ºC (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Chicken Newcastle Disease virus Antibody (NDV-Ab). High Dose Hook Effect is due to excess of antibody for very high concentrations of Chicken Newcastle Disease virus Antibody (NDV-Ab) present in the sample.
3. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Chicken Newcastle Disease virus Antibody (NDV-Ab). It is recommended that all Controls and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to poor sensitivity of the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Bring the kit at room temperature before use. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates.

2. Label the sample wells, Negative Control, Positive Control wells in duplicates.

3. Add **40 ul sample diluent and 10 ul sample** to the sample well.

4. **Add 50 ul Negative Control** and **50 ul Positive Control** to respective wells.

5. Cover the plate with a sealer and incubate for 30 minutes at 37°C

6. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

7. Add **50 ul HRP Conjugate** to each well, except blank well, gently tap the plate to ensure thorough mixing.

8. Cover the plate with a sealer and incubate for 30 minutes at 37°C

9. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

10. Pipette **100 ul of TMB Substrate** into each well.

11. Cover the plate with a sealer and incubate for 10 minutes at 37°C. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

12. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

13. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Cut-off Value = Mean of Negative Control + 0.15

Validity of the test:

Test is valid if the following conditions are met, if not we recommend to re-test
Mean Absorbance of Negative Control ≤0.15;
Mean Absorbance of Positive Control ≥1.00;

Interpretation of Results

Negative Sample: if the sample OD value < Cut-off Value, the Chicken Newcastle Disease virus Antibody (NDV-Ab) is Negative;
Positive Sample: if the sample OD value ≥ Cut-off Value, the Chicken Newcastle Disease virus Antibody (NDV-Ab) is Positive.

Precautions:
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

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