AffiGEN Inc.

AffiVET® ELISA

NDV Ab

AFG-VGS-40

POULTRY

POULTRY

The AffiVET® Newcastle Disease Virus Antibody Elisa Kit is used to detect Newcastle Disease Virus (NDV) Antibody in serum of poultry, to assess antibody condition by Newcastle disease virus (NDV) vaccine poultry and assist diagnosis of serological infected poultry.

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Overview

The AffiVET® Newcastle Disease Virus Antibody Elisa Kit serves as a crucial tool in the poultry industry for detecting Newcastle disease virus (NDV) antibodies present in the serum of poultry.

This advanced kit plays a pivotal role in assessing the antibody levels resulting from Newcastle disease virus (NDV) vaccination in poultry, providing valuable insights into the effectiveness and condition of the vaccination. Additionally, the kit aids in diagnosing serological infections in poultry, contributing to the overall health monitoring and disease management in avian populations.

With its precision and reliability, the AffiVET® Newcastle Disease Virus Antibody Elisa Kit emerges as an indispensable asset in safeguarding poultry health and ensuring the efficacy of vaccination strategies.

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KIT COMPONENTS

NDV Antigen Coated Microplate	1X 96 Tests	2X 96 Tests
Enzyme Conjugate	11 mL	22 mL
10X Concentrated Washing Buffer	100 mL	100 mL
Substrate	11 mL	22 mL
Sample Dilution	100 mL	100 mL
Stop Solution	15 mL	15 mL
Positive Control	1 mL	1 mL
Negative control	2 mL	2 mL
Adhesive Foil	2 PCE	4 PCE
User Manual	1 PCE	1 PCE

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TEST PRINCIPLE

1) **Coating Plate:** A microtiter plate is coated with a fixed amount of purified NDV antigen.

2) **Sample Preparation:** Serum samples from animals are collected and prepared. These samples may contain NDV antibodies.

3) **Incubation:** A portion of each serum sample is mixed with an enzyme-labeled monoclonal antibody specific to NDV antibodies.

4) **Plate Incubation:** The mixture of the serum sample and labeled antibody is added to the coated microtiter plate, allowing it to bind to the NDV antigen immobilized on the plate.

5) **Competitive Binding:** If NDV antibodies are present in the serum sample, they will compete with the labeled monoclonal antibody for binding to the NDV antigen on the plate. The degree of competition depends on the antibody concentration in the sample.

6) **Washing:** After an incubation period, the plate is washed to remove any unbound serum proteins or antibodies.

7) **Detection:** The TMB substrate is added to the plate, and if the labeled monoclonal antibody has bound to the NDV antigen due to the absence of NDV antibodies in the sample.
The blue signal by Enzyme catalysis is in inverse proportion of antibody content in sample.

8) **Color Measurement:** The intensity of the color developed is inversely proportional to the presence of NDV antibodies in the serum sample. In other words, if a sample contains a higher concentration of NDV antibodies, there will be less binding of the labeled antibody and consequently, less color development.

9) **Data Analysis:** The optical density (OD) of the colored product is measured using an ELISA reader at 450nm wavelength after adding stop solution to stop the reaction.

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MATERIAL REQUIRED
BUT NOT PROVIDED

1) Microplate Reader
96 wells with 450/630 nm wavelength.

2) Precise micropipette
10uL – 100uL & 100uL–1000uL.

3) Graduate: 500 mL.

4) Disposable pipette tips.

5) Distilled water or deionized water.

6) Bottle washer or Microplate Washer.

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SAMPLE PREPARATION

1. Take animal whole blood.
2. Prepare serum according to regular methods.

NOTE :
The serum should be clear, have no hemolysis.

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WASHING BUFFER PREPARATION

1. Return 10X Concentrated Washing Buffer into room temperature before use, if there is salt crystals.
2. Shake to make it dissolved.
3. Dilute it at 10 times with distilled water or deionized water.

NOTE :
The diluted washing buffer can store at 4℃ for about 1 week.

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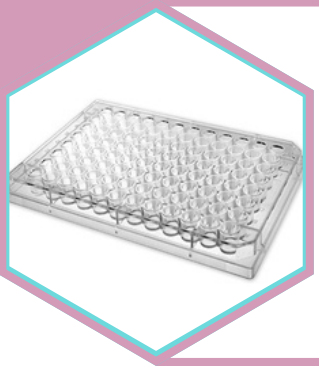
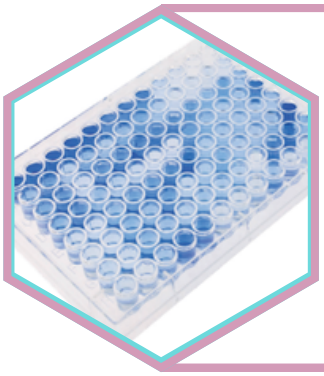
TEST PROCEDURE



Take the antigen coated plate (the plate can be open and used for several times according to sample quantity each time), for every test, set 1 well for positive control and 2 wells for negative control, positive control and negative control do not need dilute, take 100μL directly and add into its well.

Add Sample dilution to reaction wells,90μL/well, then add serum sample to the reaction wells,10μL/well, blow and mix evenly (Do not mix using tips) .



Cover it with Adhesive Foil, incubate at 37°C for 60 minutes.
Open the adhesive foil, discard the liquid of the well, add diluted washing buffer to each well, 250μL/well, then discard the liquid, repeat the above step for4-6 times, at last flap to dry with the absorbent paper.

Add Enzyme Conjugate 100 μL/well, cover it with Adhesive Foil, incubate at 37°C for 60 minutes.

Open the adhesive foil, discard the liquid of the well, washing for 4-6 times as step four , remember at last flap to dry with the absorbent paper.
Add substrate, 100 μL/well, mix it evenly then cover it with Adhesive Foil, incubate at 37°C in dark for 15 minutes.
Add stop solution 50 μL/well to stop the reaction, measure the result in 10 minutes.

RESULT INTERPRETATION

- Results Evaluation

Measure the optical density (OD) at 450 nm (with 630 nm as a reference) using an ELISA Reader.
To ensure the validity of the assay:

- **The OD value of the Negative control (N) should be ≥0.4.**

- Calculation Method

Compute the S/N value using the formula :

$$\text{S/N value} = \frac{\text{Sample OD value}}{\text{Average OD value of Negative control (N)}}$$

- Interpretation of Results

- **S/N value ≥ 0.5 indicates a Negative result.**
- **S/N value < 0.5 indicates a Positive result.**

PRECAUTIONS & WARNINGS

1) Return all reagents into room temperature before use, put the reagents at room temperature for at least 1 hour. Shake it evenly before use, and store back to 2-8℃ after usage.

2) Do not mix use reagents from different kits and different lot number, prevent the reagents been polluted when using.

3) Substrate and stop solution may have irritation to skin and eyes, be careful to use.

4) Do not expose Substrate to strong light and avoid contact with the oxidant.

5) H5 Ag coated plates should be sealed and moisture-proof. Put back unused Micro-Well plate into dry foil bag and sealed at 2-8 ℃.

6) All wastes should be treated well to avoid pollution before discarding.

7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.

8) NDV Ag Coated plates is disposable, do not repeat use.

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GET IN TOUCH

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