Newcastle Disease Virus Antibody Test Kit, ELISA

BioChek
VETERINARY DIAGNOSTICS
BioChek Poultry Immunoassays

Catalogue Code CK 116

Description of Test

The NDV ELISA kit will measure the amount of antibody to NDV in the serum of chickens. Microtitre plates have been pre-coated with inactivated NDV antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-NDV antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-NDV antibodies bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-NDV antibody is present and the intensity is related to the amount of anti-NDV antibody present in the sample.

Reagents provided:

1. **NDV Coated plates.** Inactivated viral antigen on microtitre plates.
2. **Conjugate reagent.** Anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v).
3. **Substrate tablets.** pNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer reagent.** Diethanolamine buffer with enzyme co-factors.
5. **Stop solution.** Sodium Hydroxide in Diethanolamine buffer.
6. **Sample diluent reagent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v).
7. **Wash buffer sachets.** Powdered Phosphate Buffered Saline with Tween.
8. **Negative control.** Specific Pathogen Free serum in Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v).
9. **Positive control.** Antibodies specific to NDV in Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v).

Materials and Equipment required (not provided with kit):

- Precision Pipettes and disposable tips
- 8 or 12 channel pipette/repeater pipette
- Plastic tubes for sample dilution
- Distilled or deionised water
- Microtitre Plate Reader with 405 nm filter
- Microtitre Plate Washer

Warnings and Precautions:

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation:

1. **Substrate Reagent.** To make substrate reagent, add 1 tablet to 5.5 - 6 ml of substrate buffer and allow to mix until fully dissolved (+/- 10 minutes). The prepared reagent should be made on day of use but will be stable for one week if kept in dark at +4 °C. Drop tablets into clean container and add appropriate volume of substrate buffer. **DO NOT HANDLE TABLETS WITH BARE FINGERS**
2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing.
3. All other kit components are ready to use but allow them to come to room temperature (22-27°C) before use.

Sample preparation:

1. Dilute each test sample 1:500 in Sample diluent reagent.
2. Take 5ul of sample and pipette into dilution plate recording the position of each sample on a template.
3. Add to these wells 245ul of Sample diluent reagent.
4. Take 25ul of this dilution and add to a second dilution plate.
5. Add to these wells 225ul of Sample diluent reagent to give 1:500 dilution for addition to test plate.

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING!!

Test procedure:

1. Remove NDV coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1.
3. Add 100 µl of positive control into wells C1 and D1.
4. Add 100 µl of diluted samples into all the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (350µl per well). Invert plate and tap firmly on absorbent paper until no moisture is visible.
6. Add 100 µl of Conjugate reagent into all the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Aspirate contents of wells and wash 4 times with wash buffer (350µl per well). Invert plate and tap firmly on absorbent paper until no moisture is visible.
8. Add 100 µl of Substrate reagent into all the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.
Results:

For the test to be valid the mean negative control optical density should read below 0.30. Additionally, the difference between the mean negative control and the mean positive control should be greater than 0.15.

Variance in lab temperatures will lead to lower or higher optical density values. Test sample values will be relative to the control values and the test will still be valid.

The NDV positive control has been carefully standardised to represent significant amounts of antibody to NDV in Chicken serum. The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio).

Interpretation of results:

Samples with an S/P of 0.35 or greater contain anti-NDV antibodies and are considered POSITIVE.

1. Calculation of S/P ratio:

\[
\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = S/P
\]

2. Calculation of Antibody Titre:

The following equation relates the S/P of a sample at a 1:500 dilution to a titre.

\[
\text{Log10 Titre} = 1.0 \times \log (\text{SP}) + 3.52
\]

\[
\text{Antilog} = \text{Titre}
\]

<table>
<thead>
<tr>
<th>S/P value</th>
<th>Titre Range</th>
<th>Antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.349 or less</td>
<td>1158 or less</td>
<td>Negative</td>
</tr>
<tr>
<td>0.350 or greater</td>
<td>1159 or greater</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Each Laboratory should establish its own criteria for immunity with respect to antibody titre based on correlation of BioChek NDV to current laboratory test methodologies (i.e. HI and SN) and on historical antibody responses to specific vaccines and vaccination protocols.

BioChek has available a software programme which can be used with the NDV kit to calculate S/P values, titres and provide general flock profiling.

Manufacturer: BioChek (UK) Ltd.
11 Mill Farm Business Park
Hounslow, Middlesex
London TW4 5PY

Distributor: Diagnostic Kits Imported by: BioChek, USA.
3 Southgate Road, Building 1, Unit 2
Scarborough Maine 04074
US Permit No. 588A
E-mail: info@biochek.com
Website: www.biochek.com

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