Avian Influenza Antibody Test Kit

BioChek Immunoassays

Product Code CK121

Description of test

The Avian Influenza Antibody Test Kit (AI) will measure the amount of antibody to AI in the serum of chickens. Microtitre plates have been pre-coated with inactivated AI antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-AI 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-AI 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-AI antibody is present and the intensity is related to the amount of anti-AI antibody present in the sample.

The validation data for this kit have been certified by the OIE, based on expert review, as fit for the following purposes:
Serological diagnosis of type A avian influenza in chickens (specific to IgG in serum) and for the following purposes:

1. *To demonstrate historical freedom from infection in a defined population (country/zone/compartment/flock);*
2. *To demonstrate re-establishment of freedom after outbreaks in a defined population (country/zone/compartment/flock);*
3. *To confirm diagnosis of suspect or clinical cases;*
4. *To estimate prevalence of infection to facilitate risk analysis in non-vaccinated populations (surveys/herd health schemes/disease control);*
5. *To determine immune status in individual animals or populations (post-vaccination)*
   (As stated in the resolution adopted by the OIE World Assembly of Delegates).

Reagents provided

1. Coated plates. Inactivated Avian Influenza 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 reagent.
4. Substrate buffer. 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 (0.2 % 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 (0.2 % w/v).
9. Positive control. Antibodies specific to AI in Phosphate buffer with protein stabilisers and sodium azide preservative (0.2 % w/v).

Materials and equipment required (not provided with kit)

Precision pipettes and disposable tips
8 or 12 channel pipette/repeater pipette
Plastic tubes or dilution plate for sample dilution
Distilled or deionized water
Microtiter plate reader with 405 nm filter
Microtiter plate washer

Warnings and precautions

1. Handle all reagents with care. Wear gloves, safety glasses and a laboratory coat. Stop solution and substrate buffer contain a high pH, therefore these strong alkalis are corrosive and can cause serious eye damage. The stop solution and substrate buffer must be handled with caution. If in contact with skin or eyes, wash with copious amounts of water for a minimum of 15 minutes and if the eye area is still irritated seek medical attention.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with a 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.
6. For veterinary use only.
7. Refer to product Safety Data Sheet for additional information.

Reagent preparation

1. Substrate reagent. To make substrate reagent, add 1 tablet to 5.5 ml of substrate buffer and allow to mix until fully dissolved (approx. 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 (Note that positive and negative kit controls do not require diluting)

Dilute each test sample 1:500 in sample diluent reagent. A 2-step dilution procedure is recommended.
Example:

1. Take 5 µl of sample and pipette into dilution plate recording the position of each sample on a template.
2. Add to these wells 245 µl of sample diluent reagent to make a 1:50 dilution.
3. Make the second dilution by adding 90 µl of sample diluent provided to the coated plate (NOTE: diluent must be added to the coated plate first in this example).
4. Take 10 µl of the 1:50 dilution of samples and add directly to the coated plate. This provides a 1:500 sample dilution on the coated plate.

Test procedure

1. Remove 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. Each sample is run in a single well. Add 100 µl of diluted 1:500 samples into 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 (350 µl per well). Invert plate and tap firmly on absorbent paper until no moisture is visible.
6. Add 100 µl of conjugate reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27 °C) for 30 minutes.
7. Repeat wash procedure as in 5.
8. Add 100 µl of substrate reagent into 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 and read the assay within 30 minutes.
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 assay to be valid the mean negative control absorbance should read below 0.30. The difference between the mean negative control and the mean positive control should be greater than 0.15.

The AI positive control has been carefully standardised to represent significant amounts of antibody to AI 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 0.5 or greater contain anti-AI antibodies and are considered positive.

1. Calculation of S/P ratio

$$\frac{\text{Mean of test sample - Mean of negative control}}{\text{Mean of positive control - Mean of negative control}} = \text{S/P}$$

2. Calculation of antibody titre

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

Log_{10} Titre = 1.1 * Log_{10} (S/P) + 3.156

Antilog = Titre

S/P value	Titre range	Antibody status
0.499 or less	667 or less	No antibody detected
0.500 or greater	668 or greater	Positive

This test is highly specific for antibodies against Avian Influenza. However, be aware that false positive reactors can occur in rare circumstances. Therefore confirmation with an established reference method is required for a final diagnosis.

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

KI/CK121REV05

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