

Flu DETECT™ BE

KIT FOR THE DEMONSTRATION OF SPECIFIC ANTIBODIES AGAINST AVIAN INFLUENZA VIRUS IN SERA AND PLASMAS (INDIVIDUAL)

BLOCKING IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. INTRODUCTION

Influenza type A viruses can infect avian, porcine, equine and other species including human. Sixteen serologically distinct hemagglutinin and nine neuraminidase subtypes of influenza type A virus have been isolated from avian species. Subtypes H5 and H7 are associated with significant to catastrophic losses. Disease signs in poultry range from only a slight decrease in egg production to fulmination infections with high mortality. Signs of infection may include respiratory problems, edema of the head and face, or diarrhea.

II. INTENDED USE

Flu DETECT™ BE is an in-vitro immunoassay designed to aid in the qualitative detection of antibody to Avian Influenza virus (AIV). This assay detects all 16 subtypes of Avian Influenza. Positive results should be submitted to a reference lab for confirmation and subtype determination. Negative results indicate that no detectable Influenza Type A antibody is present.

III. PRINCIPLE OF THE TEST

The Flu DETECT™ BE kit uses an immunoenzymatic technique allowing the detection of anti- Avian Influenza virus antibodies in serum and plasma samples.

The reaction is composed of three steps:

- Each serum or plasma sample is placed in a well sensitised with the viral nucleoprotein (NP) antigen. Antibodies present in the sample bind to the antigen coated plate.
- After tapping out the liquid from each well, an HRP-conjugated Anti-AIV antibody is added. This monoclonal antibody, which binds to the viral nucleoprotein (NP) antigen specific epitope, fixes onto the free antigenic sites forming a complex:
(Ag) - (Mab anti-viral nucleoprotein (NP) antigen / peroxidase).
- Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by the addition of a substrate which is transformed into a coloured product (greenish color). The corresponding optical densities are read at a single wavelength of 405 or 410 nm :
 - In the absence of antibodies in the sample, a coloured reaction is observed due to the reaction of the enzyme conjugate, which is bound to the free antigen fixed to the solid support.
 - In the presence of anti- Avian Influenza virus antibodies in the sample, less enzyme conjugate is bound to the antigenic sites on the solid support and thus the coloured reaction is diminished.

IV. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
4 microplates containing 12 strips of 8 wells sensitised with viral nucleoprotein (NP) antigen	Use within 4 weeks after opening of the sachet which must be closed after use.
Conjugate: viral nucleoprotein (NP) antigen specific MAb - peroxidase (CJ) (100 X concentrated)	Dilute 100 times in the Dilution Buffer and use within 2 h after dilution.
Buffered peroxidase substrate (ABTS)	Ready-to-use.
Negative control (NC)	Ready-to-use
Positive control (PC)	Ready-to-use
Wash solution (W) (20X concentrated)	Dilute 20 times in distilled or demineralised water. Use within 48 hrs after dilution.
Dilution Buffer (DB) for sample and conjugate dilution	Ready-to-use.
Stop solution (S) (5X concentrated)	Dilute 5 times in distilled or demineralised water. Use within 48 hrs after dilution.
Adhesive films	12 films

Note : Store diluted reagents at +5°C ± 3°C and use as mentioned above.

V. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 10 to 1000 µl. Measurement deviation must be ≤ 10% for volumes ≤10 µl and ≤ 5% for all other volumes.
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for monochromatic reading at 405 or 410 nm.

VI. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

- Do not mix or associate reagents from kits with different batch numbers
- Do not use reagents after the expiry date.
- Allow all reagents to reach laboratory temperature. Caution: only the reagents to be used in the following step are concerned.
- Handle all reagents and samples as biohazardous material.
- Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
- Never pipette by mouth.
- Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
- Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
- It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk phrases :

- R 23/25 : Toxic by inhalation and if swallowed.
R35 : Causes severe burns.
R 36/37/38 : Irritating to eyes, respiratory system and skin.
R 41 : Risk of serious damage to eyes.
R 42/43 : May cause sensitization by inhalation and skin contact.
S 7 : Keep container tightly closed.
S 24 : Avoid contact with skin.
S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S30 : Never add water to the product.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.

VII. SAMPLES

For routine serologic flock monitoring, it is suggested that at least 30 or more sera per flock be randomly collected at standard time intervals (i.e. every four weeks). **To achieve better specificity and to minimize possible false positive reactions, serum samples that are contaminated with bacteria or are very fatty should be excluded from the testing.**

The reaction is performed on individual sera and plasma diluted at 1:4. Samples should be stored as follows to provide reliable test results:

Samples	Cold (+ 2 to +8°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Individual serum and plasma	max. 4 days	Yes	No

VIII. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each plate or partially used plate.

A. PRELIMINARY STEPS

Carefully set up the distribution and identification of controls and samples.

1. Preparation of serum samples

For larger number of samples:

- Dispense minimum 100 µl of sample in a clean, tissue culture 96 well microtiter plate. Leave wells A1 to A4 empty. Frozen serum samples should be completely thawed and thoroughly mixed before diluting.

2. Preparation of Conjugate Solution

HRP-Conjugated anti-NP Antibody is supplied as a stock solution. Dilute 100 µl of stock conjugate solution in 10 ml Dilution Buffer (1:100 dilutions). **Mix Well.** This 10 ml preparation will supply sufficient conjugate for one 96 well ELISA plate.

3. Preparation of 1X Wash Solution

Dilute 25 ml concentrated Wash Solution in 475 ml laboratory grade (distilled or R.O.) water (1:20). **Mix Well.**

Approximately 500 ml Wash Solution is needed for each 96 well plate.

4. Preparation of Substrate solution

Substrate solution is ready to use. Each Plate will require approximately 10 ml substrate solution.

5. Preparation of 1X stops solution

Dilute 2.5 ml concentrated stop solution in 10 ml laboratory grade (distilled or R.O.) water 1:5. **Mix well.**

Approximately 12.5 ml stop solution is needed per 96 well ELISA plate.

Note: Storage of 5X Stop Solution at refrigerated temperatures may cause the formation of a white solid. This does not affect product performance. Warm at room temperature or 37°C to dissolve before use.

B. TEST PROCEDURE

I - SAMPLE AND CONTROL DISTRIBUTION

1. Sample distribution:

Frozen serum samples should be completely thawed and thoroughly mixed before diluting.

- Add 75 µl Dilution Buffer per well of the test plate except to wells A1 to A4
- Add 25 µl unknown serum per well (yields a 1:4 dilution)*. Start with well A5 and end with well H12. Transfer of samples to the test plate should be done as quickly as possible.
- Mix by gentle shaking the plate manually

*For larger number of samples:

Using an 8 or 12 channel pipette transfer 25 µl per well of each of the unknown serum from the uncoated 96 well microtiter to the corresponding wells of the test plate (VIII.A.1.). Discard pipette tips after each row of sample is transferred. Transfer of samples to the test plate should be done as quickly as possible.

2. Control distribution:

- Dispense 100 µl of negative control (NC) to wells A1 and A2, and 100 µl positive control (PC) to wells A3 and A4.

- Strips should always be placed on the frame so that both washer and reader can be used.

- Cover the wells with adhesive film, cut to the necessary length by the number of strips used.

- Mix by gentle shaking the plate manually.

3. Incubation of the plate:

- Incubate 1 hour at room temperature 23°C (± 3°C)

Tap out the liquid from each well into an appropriate vessel containing bleach or other decontamination agent.

DO NOT WASH THE PLATE

II – ADDITION OF CONJUGATE

1. Distribution of conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

2. Incubation of conjugate:

Incubate 30 minutes at room temperature 23°C (± 3°C).

WASHING:

Carefully remove the adhesive film, tap out the liquid from each well into an appropriate vessel containing bleach or other decontamination agent and wash **5** times manually or with a comparable automatic washing device.

Washing manually: Using an 8 or 12 channel pipette, fill each well with approximately 300 µl Wash Solution. Discard contents into an appropriate waste container **without soak cycle** (waste container should contain bleach solution). Tap inverted plate to ensure that all residual liquid is removed. **Repeat wash procedure 4 more times.**

Sufficient and homogenous washing of the plate is absolute crucial to afford valid results. The use of an automated plate washer or a wash bottle with spout tube is highly recommended.

III – REVELATION

1. Addition of the substrate:

Add 100 µl of buffered peroxidase substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually.

2. Incubation of substrate:

15 min at room temperature 23°C (± 3°C), shielded from light.

3. Addition of Stop Solution:

Add 100 µl of diluted stop solution per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells.

4. Measure of the optical density:

Measure the optical density (OD) monochromatically at 405 or 410 nm (in the green band).

The ELISA plate reader should be blanked as directed.

IX. TEST VALIDATION

Valid *Flu* DETECT™ BE result is obtained when:

- the average optical density (O.D) value of the Negative controls (A1, A2) is 0.500 +/- 0.15.

Under optimal conditions* the suggested O.D. value range of 0.65 and 0.35 should be strived for Negative Controls to ensure the most consistent laboratory results. Please note that tests with O.D. values which do not fall within the suggested range above do not constitute invalid test.

- The S/N ratio of the average optical density (O.D.) value of the Positive controls (A3, A4) is less than 0.6

This S/N ratio is calculated as follows:

$$\text{S/N ratio} = \frac{\overline{\text{OD}} \text{ PC}}{\overline{\text{OD}} \text{ NC}}$$

$\overline{\text{OD}}$ = average of the optical densities of the negative and positive control.

*Optimal conditions are at room temperature (21°C to 24° C) Higher room temperatures may result in slightly higher O.D. values.

X. EXPRESSION AND INTERPRETATION OF THE RESULTS

- Divide the sample O.D. reading by the average Negative O.D. reading for Sample/Negative ratio (S/N).
- S/N ratio greater or equal than 0.6 is negative and less than 0.6 is positive.

This S/N ratio is calculated as follows:

$$\text{S/N ratio} = \frac{\text{O.D. reading for Sample Absorbance}}{\overline{\text{OD}} \text{ NC}}$$

$\overline{\text{OD}}$ = average of the optical densities of the negative control.

Example:

- Example Negative Controls:

O.D. duplicates negative controls: 0.602 and 0.589,

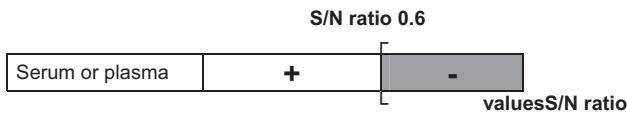
Average O.D. negative controls= (0.602+0.589)/2 = 0.595

- Example S/N value calculation:

O.D reading for Sample Absorbance = 0.424

S/N ratio = 0.424 / 0.595 = 0.712

RESULTS



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