# Chicken IgY (IgG) ELISA Test: An Enzyme-Linked Immunosorbent Assay for the Detection of Chicken Antibodies in Egg Yolk or Blood Serum

## Product Insert

**Cat. No. ED209-2 (2 x 96 wells)**

**For Research Use Only**

### Introduction

An **Enzyme-Linked Immunosorbent Assay (ELISA)** is an immunochemical technique that can be used to detect the presence of **antigens** (Point of Care Flu tests, pregnancy tests, etc.) or **antibodies** (HIV-1, Lyme disease, Autoimmune disease, etc.) in a sample. In broad terms, an antigen is a material that is foreign to an animal's immune system and therefore elicits an immune response. Many different materials can serve as antigens. For example, macromolecules such as proteins, polysaccharides, and nucleic acids often serve as antigens. In fact, it is usually these individual components of complex microorganisms that stimulate immunity during a natural infection (Influenza A) or vaccination (Flu vaccine).

A major and vital component of the immune response to antigens is the production of a family of proteins called antibodies ("anti-foreign bodies") that are classified as immunoglobulins (IgG, IgM, IgA, IgD, IgE). Antibodies are complex proteins with many roles to play in the immune response, but one of the key roles is the capability to very specifically attach to or bind to the antigen that stimulated its production. This is very similar to the fit of a key (antigen) into the appropriate lock (antibody). The attachment is very specific and very strong. For example, antibodies produced in response to a tetanus vaccination will not react with (and provide protection against) influenza A virus, and likewise, antibodies produced as a result of influenza A vaccination are not protective against the tetanus toxin produced by the bacterium *Clostridium tetani*.

Under the appropriate circumstances, **antibodies can also be antigens** and this phenomenon permits the generation of very specific reagents that can be used to produce immunological tests. For example, while antibodies produced by many different species are similar in structure and function, they exhibit species to species differences. As a result, injecting human antibodies (now the antigen) into a goat or a rabbit elicits an immune response and goat or rabbit anti-human antibodies will be produced. Likewise, inoculation of goats or rabbits with chicken antibodies (now the antigen) will result in the production of anti-chicken antibodies. While antibodies produced by all species are released into the blood, bird antibodies are also found in egg yolks. As a result, these antibodies are often referred to as "IgY" antibodies, but they are essentially the bird version of IgG antibodies found in other species.

Because of the high concentration of IgY found in egg yolks, eggs are a convenient and safe source of antibodies for study and to demonstrate the power off the ELISA technique. Virusys has developed an easy to use ELISA that permits the sensitive detection and quantitation of chicken IgY in egg yolks. The assay is based on the "antigen capture" technique:

1. Purified rabbit anti-chicken IgY ("capture" antibody) is attached to the surface of a plastic well (supplied as 96-well ELISA plates).
2. A sample that may or may not contain chicken IgY is then added and allowed to incubate for 15 min. If present in the sample, the chicken IgY binds to or is "captured" by the rabbit anti-chicken IgY, while other macromolecules in the sample are not (lock and key concept).
3. The sample is then removed from the well and the well is washed to remove residual sample components.
4. Purified goat anti-chicken IgY is then added to the well (detection antibody) and incubated for 15 min. This material not only will bind to chicken IgY (if it was captured from the sample), but it contains an additional modification; chemically attached to the goat anti-chicken IgY protein is an enzyme (Horseradish Peroxidase; HRP). If IgY was captured from the sample, then the detection antibody (goat anti-chicken IgY-HRP) will bind to the captured IgY in an amount that is proportional to the captured IgY.
5. The unbound detection antibody is then removed and the well is washed again. This is a very important wash step, because it is necessary to remove unbound detection antibody (and therefore HRP), from the well.
6. Chromagen is then added to the well. The enzyme reaction will convert the chromagen (enzyme substrate) to a colored (blue) reaction product. The amount of reaction product that is produced is directly proportional to the amount of goat anti-chicken IgY-HRP in the well, which in turn, is proportional to the amount of chicken IgY that was captured from the sample.
7. Since the enzyme reaction will continue as long as chromagen is available, a stop reagent is added to the well after 15 min. that inhibits the enzyme, changes the blue color to yellow, and stabilizes the yellow color.
8. The yellow reaction product can then be quantified visually or using an instrument.

### Kit Components

1. IgY Antigen Capture Plate (96 tests)-2 ea.
2. IgY Sample Diluent (1x)-2 x 50 ml
3. IgY Standard (10 ug/ml)-0.1 ml
4. Wash Buffer (20x)-2 x 50 ml
5. IgY Detection Antibody, HRP-labeled (1x)-24 ml
6. Chromagen Solution (1x)-24 ml
7. Stop Solution (1x)-24 ml

## Materials Needed but Not Supplied

1. Chicken Eggs
2. Egg Yolk Diluent (IGY-4001)
3. Microspin Filter Units (0.45 u) or Syringe Filters
4. Balance
5. Plastic weigh boats
6. Adjustable multichannel pipettor (8 or 12 tip, 20-300 ul)
7. Adjustable pipettors (20-200 ul, 200-1000 ul)
8. Pipette tips
9. Plastic pipettor reservoirs
10. Serological pipettes (5 ml, 10 ml)
11. Glass tubes (13 x 100 mm)

## Storage

Store all kit components at 2-8° C. Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8° C. The crystals can be re-dissolved by swirling the bottle in warm tap water.

## Procedure

1. Remove the kit components from storage and allow them to warm to room temperature.
2. Prepare **1x Wash Buffer** by diluting and mixing the contents of one bottle of **20x Wash Buffer** with 950 ml of distilled or deionized water.
3. While the kit components are warming, process one or more egg yolks for testing according to the instructions supplied with the **Egg Yolk Diluent (IGY-4001)**. Once processed, the clarified egg yolk should be diluted 1:100 ($10^{-2}$), followed by serial 10-fold dilutions as shown in the chart below:

| Dilution | Egg Yolk (ul) | Sample Diluent (ul) |
|---|---|---|
| $10^{-2}$ | 10 ul processed yolk | 990 ul |
| $10^{-3}$ | 100 ul @ $10^{-2}$ | 900 ul |
| $10^{-4}$ | 100 ul @ $10^{-3}$ | 900 ul |
| $10^{-5}$ | 100 ul @ $10^{-4}$ | 900 ul |
| $10^{-6}$ | 100 ul @ $10^{-5}$ | 900 ul |
| $10^{-7}$ | 100 ul @ $10^{-6}$ | 900 ul |
| $10^{-8}$ | 100 ul @ $10^{-7}$ | 900 ul |
| $10^{-9}$ | 100 ul @ $10^{-8}$ | 900 ul |

4. To generate a standard curve, the **IgY Standard** must be diluted 1:100 to generate a value of 100 ng/ml. Serial 2-fold dilutions are then prepared from the first dilution as shown in the chart below:

| IgY (ng/ml) | IgY Standard (ul) | Sample Diluent (ul) |
|---|---|---|
| 100 | 10 ul @ 10 ug/ml | 990 ul |
| 50 | 500 ul @ 100 ng/ml | 500 ul |
| 25 | 500 ul @ 50 ng/ml | 500 ul |
| 12.5 | 500 ul @ 25 ng/ml | 500 ul |
| 6.25 | 500 ul @12.5 ng/ml | 500 ul |
| 3.13 | 500 ul @ 6.25 ng/ml | 500 ul |
| 1.56 | 500 ul @ 3.13 ng/ml | 500 ul |
| 0 | ---------- | 500 ul |

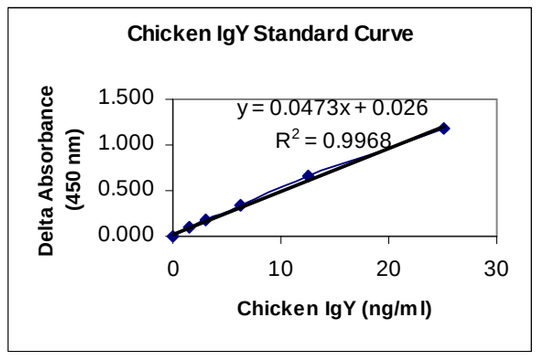
5. Determine the number of test wells needed. Use one well for each sample. In addition, include one well for each dilution of the **Standard Curve.** Unused strips and dessicant may be returned to the foil pouch and sealed for future use.
6. To begin the assay, transfer 100 ul of each standard and sample to the appropriate wells of the **IgY Antigen Capture Plate**.
7. Incubate for 15 min. at room temperature.
8. Empty the wells by flicking the contents into a sink and then wash the wells 5x with **1x IgY Wash Buffer**. Washing can be accomplished by using an automated washer (if available), by using a squeeze bottle, or by alternately filling the wells with a multi-channel pipettor followed by flicking out the wash buffer.
9. Add 100 µl of **IgY Detection Antibody** to each well and incubate for 15 min.
10. Wash by repeating Step 8.
11. Add 100 ul of **IgY Chromagen** to each well and incubate for 15 min.
12. Stop the reaction by the addition of 100 µl of **IgY Stop Solution**.
13. Tap the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

## Quality Control

1. Absorbance value for 0 ng/ml Standard should be ≤0.250.
2. Absorbance value for 100 ng/m Standard should be ≥2.000.

## Determination of IgY Concentration (ng/ml) in Samples

1. To determine the concentration of chicken IgY in individual samples, it is first necessary to subtract the absorbance value obtained for the 0 ng/ml Standard from all other Standard absorbances and sample absorbances.
2. Prepare a standard curve by plotting the corrected absorbance value for each of the following standards on the y-axis versus the concentration of IgY (ng/ml) for that standard (25, 12.5, 6.3, 3.1, 1.6, and 0) on the x-axis. Sample IgY concentrations can be obtained by interpolation and dilution factor multiplication.
3. If using software (Microsoft Excel, for example), perform linear regression and record the $R^2$ value (should be >0.95) and the equation. Use the equation and dilution factor to calculate the concentration of IgY in the samples.

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