PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of Chicken IgY were mixed into each of 2 serum and 1 yolk sample. Observed assay values compared to expected values ranged from 92 to 102%, indicating accurate quantification of IgY in chicken serum and yolk.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgG Concn		40.85	
+ Chicken serum A, 30.8 ng/ml	71.6	72.8	**102%**
+ Chicken serum B, 17.2 ng/ml	58.0	56.1	**97%**
+ Chicken yolk 1, 30.7 ng/ml	71.6	70.6	**99%**
Low IgG Concn		9.05	
+ Chicken serum A, 30.8 ng/ml	39.8	38.6	**97%**
+ Chicken serum B, 17.2 ng/ml	26.2	24.2	**92%**
+ Chicken yolk 1, 30.7 ng/ml	39.8	38.2	**96%**

ELISA Kit Components	Amount	Part No.
Anti-Chicken IgY (IgG) Microwell Strip Plate	8-well strips (12)	6031
Chicken IgY (IgG) Control	0.65 ml	6032
Chicken IgY (IgG) Standard 10 ng/ml	0.65 ml	6033B
Chicken IgY (IgG) Standard 20 ng/ml	0.65 ml	6033C
Chicken IgY (IgG) Standard 50 ng/ml	0.65 ml	6033D
Chicken IgY (IgG) Standard 100 ng/ml	0.65 ml	6033E
Chicken IgY (IgG) Standard 200 ng/ml	0.65 ml	6033F
Anti-Chicken IgY (IgG) HRP Conjugate (100X)	0.15 ml	6034
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-6030

Instruction Manual No. M-6030

Chicken IgY (IgG)

ELISA Kit Cat. No. 6020/6030

For Quantitative Determination of IgY (IgG) In Chicken Serum or Egg Yolk

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INTENDED USE

The Chicken IgY ELISA Kit is an in vitro immunoassay for research use in the quantification of Chicken IgY circulating in serum, in egg yolk, or in other appropriately qualified samples from tissue fluids (e.g., mucosa, egg white), or in cultures of chicken cells.

RESEARCH USE OF THE TEST

Chickens and other avian species produce a unique immunoglobulin molecule called IgY. IgY is the accepted/proper term for chicken antibodies, the 'Y' indicating that the immunoglobulin is distinctly different from mammalian IgG, although funtionally equivalent. Produced in the serum of chickens, IgY is passed from the mother (dam) to the embryo via the egg yolk, imparting a high concentration of IgY to the developing embryo. The egg yolk can yield over 100mg of IgY and is, for research and manufacturing purposes, a simple, convenient and non-invasive method for obtaining specific antibodies from immunization.

The IgY molecule has the same general structure as mammalian IgG with 2 heavy chains (~65-70 kDa) and 2 light chains (22-30 kDa) with about 3% carbohydrate. However, the heavy chain "Fc" domain of IgY is different from mammalian IgG and cannot fix complement or bind to protein A or G (thus, Protein A/G cannot be used to directly purify or immunoprecipitate IgY). Since chicken antibodies also do not cross-react with mammalian IgGs, bind to Fc receptors, interact with rheumatoid factors or react with human anti-murine antibodies (HAMA), non-specific binding of chicken antibodies is greatly reduced in most immunological assays.

PRINCIPLE OF THE TEST

The Chicken IgY ELISA kit is based on the binding of Chicken IgY in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IgY present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgY in samples and control is calculated from a curve of standards containing known concentrations of IgY.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the kit label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with serum and egg IgY (IgG), and have essentially no reactivity with IgM or any other chicken serum or egg proteins.

Serum from the following species were tested at 1:1000 dilution; turkey showed some reactivity, and the others showed no significant reactivity: goose, pigeon, duck, FBS, bovine, goat, monkey, guinea pig, sheep, pig, mouse, rat, human, hamster, and rabbit .No reactivity was seen with goose, quail or Arecan chicken egg yolk at 1:1000 dilution.

Normal Range

A limited testing of 5 adult chicken sera gave values of 3.2 – 26.9 mg/ml (average 7.1 mg/ml). Testing of 5 chicken egg yolk samples (unrelated to serum samples) gave values of 10.1 - 16.8 mg/ml (average 12.2 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgY were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations (CVs) were calculated for the concentrations using a point-to-point curve-fitting program. IgY concentrations were measured with very good within-assay (2.6 to 7.0 %CV) and between-assay (5.2 to 8.8 %CV) reproducibility.

Sample	IgG ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	28	3.7	2.5
Mid Sample	65	3.4	4.6
High Sample	101	4.8	3.3

Linearity of Dilution

Four (4) individual stored sera and yolk samples were diluted to 2 levels for testing, and concordance of the assay values was compared. Agreement of values ranged from 94 to 100%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Serum 1	1:50k 1:400k	140 18	7.0 7.2	**99 %**
Serum 2	1:200k 1:1600k	142 15.8	28.5 25.3	**94 %**
Serum 3	1:100k 1:800k	149 18.8	14.9 15.0	**100 %**
Yolk 1	1:100 1:400	141 35.4	14.1 14.2	**100 %**

CALCULATIONS

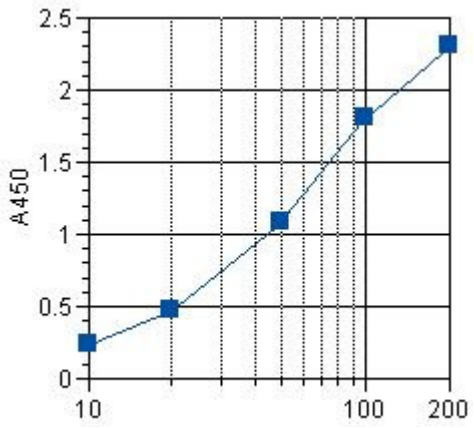
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Chicken IgY concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Chicken IgY (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Chicken IgY concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Chicken IgY ng/ml
1A, B	**Negative Diluent Control**	0.03	0
1C, D	10 ng/ml **Standard**	0.24	10
2E, F	20 ng/ml **Standard**	0.47	20
3G, H	50 ng/ml **Standard**	1.08	50
2A, B	100 ng/ml **Standard**	1.80	100
2C, D	200 ng/ml **Standard**	2.30	200
2E, F	**Positive Serum Control** [Value: 20 - 38 ng/ml]	0.69	29
2G, H	**Sample** [Diluted 1:100k]	1.31	67
	Calculated: 100k-fold dilution x 67 ng/ml = **6.7** mg/ml in serum		



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Chicken IgY (IgG) Microwell Strip Plate	6031	8-well strips (12)	Coated with purified anti-Chicken IgY antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [IgY] range on label	6032	0.65 ml	Chicken serum with stated IgY concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
Chicken IgY (IgG) Standards			
10 ng/ml	6033B	0.65 ml	Five (5) vials, each containing chicken serum calibrated using purified chicken IgY; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
20 ng/ml	6033C	0.65 ml	
50 ng/ml	6033D	0.65 ml	
100 ng/ml	6033E	0.65 ml	
200 ng/ml	6033F	0.65 ml	
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Chicken IgY (IgG) -HRP Conjugate Concentrate (100x) Part No. 6034, 0.15ml	Peroxidase conjugated anti-Chicken IgY antibody in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Antibody-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Serum, egg yolk and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. The Sample Diluent is formulated for proper dilution of egg yolk as well as serum.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **egg yolk**, separate the yolk from the egg white, then puncture the yolk and drain out the inside, being careful not to add the membranes. A two-fold dilution can be made in PBS to reduce viscosity; further dilutions must be made in Sample Diluent.

For **all samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , store refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgG concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:100k-1:800k are appropriate for most normal or immunized chicken sera or yolks. For accuracy, three dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:100] + 990ul diluent = [1:10k],
3) 50ul [1:10k] + 950ul diluent = **[1:200k].**

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, to include 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of Working Anti-Chicken IgY-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.