Chicken Egg Ovalbumin ELISA PROCEDURE SUMMARY

Total Assay Time - 75 min. (60+15)

	Allow **all** reagents to reach room temp.; arrange and label required # of strips. Dilute sample diluent 1:20, and wash buffer 1:100 with water. Dilute antibody-HRP conjugate 1:100 with 1X sample diluent. Dilute egg samples (~100K) with 1x sample diluent. **Do not dilute standards**.
Step 1	Pipet **20 ul** of pre-diluted **standards** and diluted samples (~100K) into appropriate wells. Dispense **80 ul** of antibody-enzyme conjugate to each well. Mix gently, cover the plate and incubate for **60 min** at room temp
Step 2	Aspirate and **wash 5 times** with 1x wash solution.
Step 3	Dispense **100 ul of TMB substrate Solution**. Mix gently, cover the plate and incubate for **15 min** at room temp. Blue color develops
Step 4	Pipette 100 ul stop solution (1N H2SO4) into each well. Blue color turns yellow. Measure Absorbance at 450 nm.

CHECK LIST (Check each box after completing each of the above steps)

	Step 1	Step2	Step3	Step4
Start time End Time				

KIT PROFILE

Date received: **Cat #** 6010 **Lot #** _____ **Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: **# Strips used** _ **# Remaining** _____

Date used: **# Strips used** _ **# Remaining** _____

Remarks _____

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_____

Instruction Manual No. M-6010

Chicken Egg Ovalbumin

ELISA Kit Cat. # 6010

For Quantitative Determination of Ovalbumin in Chicken Egg White

ALPHA DIAGNOSTIC INTERNATIONAL

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Chicken Egg Ovalbumin ELISA KIT Cat. No. 6010

Kit Components, 96 tests	Cat #
Ovalbumin coated strip plate (8 wells x 12 strips)	6011
Ovalbumin Std. A (1 ng/ml), 0.250 ml	6012
Ovalbumin Std B (10 ng/ml), 0.250 ml	6013
Ovalbumin Std C (100 ng/ml), 0. 250 ml	6014
Ovalbumin Std D (1000 ng/ml), 0. 250 ml	6015
Ovalbumin Std E (5000 ng/ml), 0. 250 ml	6016
Sample Diluent (20X), 10ml	SD-20PTB
Wash Buffer (100X), 10 ml	WB-100
Anti-Ovalbumin-HRP Conjugate (100X), 0.12 mll	6017
TMB Substrate, 12 ml	80091
Stop solution, 12 ml	80101
Instruction Manual	M-6010

Note: Extra 20X sample diluent can be purchased if required.

Introduction

Chicken egg is a major source of **Ovalbumin** (OVA) protein. A 386aa protein in chicken with an Isoelectric point of 4.6, it is known to contain two potential *N*-glycosylation sites at Asn-292 and Asn-311, while a single carbohydrate chain is attached only on the Asn-292 residue of the secreted genuine OVA. In SDS-PAGE under non-reducing conditions, OVA have an apparent molecular weight of 40,45,63 and 72kD, with the 45kD form as the main constituent. Under reducing conditions only a 45kD band is observed. Beside providing nutrition to yolk in egg its function is unknown, OVA belongs to serpin family of protease inhibitors.

OVA is one of the major allergens in chicken egg white, and is the often cause of hypersensitivity reactions to food. It serves as a suitable model allergen for studying the relationship between structure and function, because the amino acid sequence and post-translational modifications of the protein are known.

Egg allergies occur in about 0.5 percent of the population and in about 5 percent of children with allergies. Because influenza and yellow fever vaccines are both made in eggs, egg proteins (**primarily ovalbumin**) are present in the final product. Residual quantities of egg proteins found in the influenza vaccine (i.e., about 0.02-1.0 ug per dose) are sufficient to induce severe and rarely fatal hypersensitivity reactions in children with egg allergies. ADI has developed an ELISA kit detecting ovalbumin using polyclonal antibodies, which is of growing importance in standardization and characterization of allergens (ovalbumin) and as a tool for the detection and quantification of egg albumin in food products.

ADI's Ovalbumin ELISA provides is a rapid, specific and sensitive assay for measuring Ovalbumin in chicken egg white. Samples other than egg white, must be optimized to bring them within the testing range.

PERFORMANCE CHARACTERISTICS

1. Detection limit- Based on 5 replicate determinations of the zero standards, the minimum Ovalbumin concentration detectable using this assay is 1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. High dose hook effect
Ovalbumin concentrations of up to 20 ug/ml did not show any hook effect.

3. Expected Values: A limited testing of egg white extracts gave values of ~80-100 mg/ml of Ovalbumin.

4. Specificity: This kit is specific to Ovalbumin and does not show any significant reactivity to other egg proteins.

5. Species Crossreactivity
This kit is specific for chicken egg albumin or ovalbumin. It has not reactivity with chicken serum albumin. ELISA kits cross reactivity was tested with the following bird egg white extracts (ovalbumin(at dilutions of 1:10K: Turkey (16%) and Quail (24%). Other birds not tested.

Other ELISA kits are available from ADI (complete list at the web site)

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).
Monkey: IgM, IgG, IgA, CRP
Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF,
Chicken: IgG, IgM, IgY, Ovalbumin
Rabbit: CRP, IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM,
Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG
Sheep: IgG
Turkey: IgG

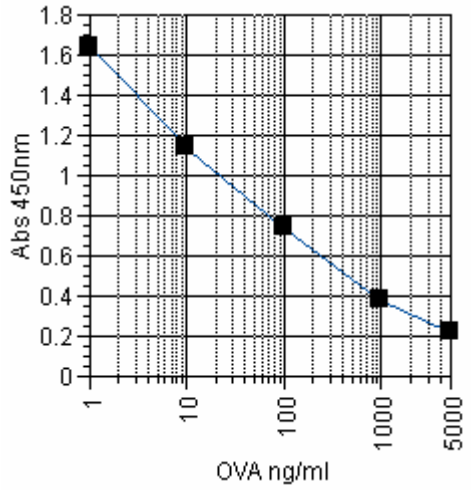
For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	*Mean $A_{450\ nm}$	Calculated Concn
A1, A2	(0.0 ng/ml)	1.79	
B1, B2	**Std. A** (1 ng/ml)	1.64	
C1, C2	**Std. B** (10 ng/ml)	1.14	
D1, D2	**Std. C** (100 ng/ml	0.74	
E1, E2	**Std. D** (1000 ng/ml)	0.38	
F1, F2	**Std. E** (5000 ng/ml)	0.22	
G1, G2	**Sample (1:100K)**	0.36	(880ng/ml) adjusted for sample dilution (88 mg/ml)

*=Average duplicate values

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

The Ovalbumin ELISA kit is based on competitive binding of fixed concentrations of anti-Ovalbumin-HRP conjugate to Ovalbumin coated on the plate and to Ovalbumin in the samples. Higher concentrations of Ovalbumin in the samples reduce the amount of anti- Ovalbumin -HRP that binds to the plate. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is indirectly proportional to the amount of Ovalbumin present in the sample, the higher the concentration of Ovalbumin in the sample the lower the absorbance will be. Adding stop solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of Ovalbumin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000ul) and multi-channel pipette with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Alpha Diagnostic International Ovalbumin ELISA kit is intended for *in vitro research* use only. The reagents contain Thermerosal (0.1%) as preservative; necessary care should be taken when disposing solutions. All other precautions must be taken to handle biological material.

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid) and dispose of it accordingly.

SPECIMEN COLLECTION AND HANDLING

Egg white is gelatinous and difficult to pipette, therefore take 0.5ml with a wide mouth pipette tip and dilute with 4.5 ml of 1X sample buffer, mix well this will be 1:10 stock. The stock can be stored at 4oC for a week or frozen in suitable aliquots at –20oC. Samples that have been heated are not suitable as it precipitates the protein. If egg white can not be immediately assayed , it could be stored at $-20^{O}C$ for up to six months. Sample dilutions other than egg white, must be optimized to bring them within the testing range. Avoid repeated freezing and thawing of samples. No preservatives should be added to the egg sample.

REAGENT PREPARATION

1. 1X Sample Diluent

Dilute the Sample Diluent stock 1:20 with water (10 ml diluent in 190ml water). Dilute only the required reagent. Store diluted solution at 4oC for 1-2 weeks.

2. 1X Wash Buffer

Dilute the wash buffer 1:100 with water. Dilute 5ml of the stock in 500ml water. Store at room temperature for 1-2 weeks.

3. Antibody-HRP Conjugate

Dilute Antibody-HRP conjugate 1:100 with 1X sample diluent (10ul in 1ml or 100 ul in 10ml for one full plate). Prepare required volume only, do not store diluted conjugate.

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 label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Do not dilute standards. Dilute egg white samples 1:100K using 1x sample diluent. Some samples may have to be diluted more or less but 1:100K should bring most normal samples to within the testing range. If the sample is other than egg, figure out your own sample dilution .

1. Due to high sample dilution, we recommend the following diluting scheme to minimize errors.

	Sample	Diluent	Total Volume	Dilution Factor
Step 1	0.5 ml of Egg White	4.5 ml	5 ml	1:10 stock
Step 2	10 ul of 1:10 stock	990 ul	1000 ul	1:1K
Step 3	10 ul of 1:1K	990 ul	1000 ul	1:100K

Note: It is possible to adjust the sample dilution at step 3 to make 1:200K or any other dilution as necessary.

2. Label or mark the microtiter well strips to be used on the plate.

3. Pipet **20ul stds.** and diluted samples into appropriate wells.

4. **Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used keeping diluted samples. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipette.

5. Pipette **80 ul of Ab-enzyme** conjugate into **each well. Mix gently for 15-30 seconds.** Cover the plate and incubate for **60 minutes** at room temperature. **Note:** the conjugate solution must be at room temperature**.**

6. Aspirate and wash the wells 5 times with 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

7. Add **100 ul of HRP**-substrate soln. (TMB) into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops. **Note:** TMB solution must be at room temperature.

8. Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.

9. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 min after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{O}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than **5000 ng/ml** Ovalbumin should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting mean absorbance values of standards against appropriate Ovalbumin concentrations. Read off the Ovalbumin concentrations of the egg samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:100K then the values must be multiplied by 100K and results expressed as ug/ml or mg/ml.