INTENDED USE

The Alpha Diagnostics Int'l **Chicken Ovalbumin** ELISA Kit is an immunoassay for the quantitation of ovalbumin from chicken egg white. The assay is also suitable for detecting ovalbumin in samples other than egg white, such as extracts of foods, vaccines, or other products or processes, with proper control for assay compatibility.

GENERAL INFORMATION

Ovalbumin (egg albumin) is one of the major allergens in chicken egg white and is often the cause of hypersensitivity reactions to food. It serves as a model allergen, suitable 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 but rarely fatal hypersensitivity reactions in children with egg allergies. ADI has developed an ELISA kit for the detection and quantification of egg albumin in food products and is an important tool for the standardization and characterization of such allergens.

PRINCIPLE OF THE TEST

The Chicken Ovalbumin ELISA kit is based on the binding of chicken ovalbumin in samples to two antibodies, one immobilized on the microwells, and the other conjugated to biotin, which then binds to a streptavidin horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of ovalbumin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The concentration of ovalbumin in samples is calculated from a standard curve of standards prepared from purified ovalbumin.

STORAGE AND STABILITY

The microwell plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the kit box label. Stabilities of the working solutions are indicated under Reagent Preparation.

KIT CONTENTS

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 + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely.
Anti- Ovalbumin Detection Antibody Concentrate (100x) Part No. 6055, 0.15ml	Biotinylated anti-ovalbumin in buffer with protein, detergents, and BND 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.
Streptavidin-HRP Conjugate Concentrate (100x) Part No. S-HRP100, 0.15ml	Peroxidase conjugated streptavidin in buffer with protein, detergents, and BND 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.

Ready for use: Store as indicated on the labels

Component	Part No.	Amt	Contents
Anti-Chicken Ovalbumin Microwell Strip Plate	6051	8-well strips (12)	Coated with purified anti-ovalbumin antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Chicken Ovalbumin Standards			
0.1 ng/ml 0.2 ng/ml 0.5 ng/ml 1 ng/ml 2 ng/ml 3 ng/ml	6053B 6053C 6053D 6053E 6053F 6053G	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Six (6) vials, each containing purified ovalbumin; diluted in buffer with protein, detergents, and antimicrobial as stabilizers.
Positive Control [Ovalbumin] range on label	6052	0.65 ml	Solution with stated ovalbumin concentration range; diluted in buffer with protein, detergents, and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

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, Detection Antibody, and HRP.
- Grad. cylinder to dilute Wash Concentrate and Sample Diluent Concn; 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 Bromo-nitro-dioxane (BND: 0.05%, w/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 BND can be requested or obtained from the ADI website.

ASSAY DESIGN AND SET-UP

Specimen collection and handling

Egg white: Egg white is gelatinous and difficult to pipette; use a wide-mouth pipette to prepare a 1:10 stock solution (e.g., 0.5ml egg white + 4.5 ml of Working Sample Diluent; mix well). The stock can be stored at 2-8°C for a week or frozen in suitable aliquots.

Caution! Samples with very high ovalbumin concentrations, such as egg white, may produce contamination of diluents, samples, etc., without stringent handling to avoid this issue.

High blank values (A450=>0.400), poor precision, and other unexpected results may indicate ovalbumin contamination problems. This is not a problem with the kit and requires that the operator take extra steps to eliminate ovalbumin contamination from the testing environment.

Food, vaccine, other extract: Dilute prepared solutions in Working Sample Diluent at levels that bring the ovalbumin concentration within testing limits. Perform solution-only negative control testing to ensure the compatibility of the sample solution in the assay.

Assay Validation

Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism, as follows:

Recovery – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the sample ovalbumin relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions a minimum 5-fold sample dilution is usually sufficient. Serum and plasma require at least a 10-fold dilution to obtain consistent quantitation or complete antigen recovery.

Parallelism – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

Plate Set-up

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

DILUTE Samples in Working Sample Diluent; >1:500K is appropriate for most egg white samples. Dilute other sample types according to expected ovalbumin levels and/or trial testing. DO NOT dilute the Standards or Positive Control.

Plate Set-up Continued

- Determine the number of wells for the assay run. Duplicates are recommended, including 12 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.
- Prior to sample addition, add 200-300ul Working Wash Solution to each well and let stand for about 1 minute.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

Assay Procedure

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. 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.

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

- Add 100ul of diluted Anti-Ovalbumin Detection Antibody to each well.
- Incubate for **60** minutes.
- Wash wells 4 times as in step 1.

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

- Add 100ul of diluted Streptavidin-HRP Conjugate to each well.
- Incubate for **30** minutes.
- Wash wells 5 times as in step 1.

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.

CALCULATION OF RESULTS

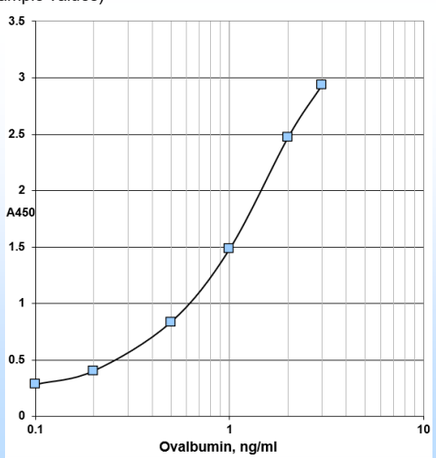
The results may be calculated using any immunoassay software package, or by plotting the data on semi-log graph paper. The four-parameter curve-fit is recommended; for hand graphing a point-to-point curve is most reliable.

The ovalbumin concentrations in unknown samples and controls can be determined by interpolation from the standard curve, and then multiplication of the values by the dilution factor to obtain ovalbumin concentration in the original prep. Samples producing signals higher than the 3 ng/ml standard should be further diluted and re-assayed.

Wells	Standards, Control & Samples		A450
A1, A2	**Diluent Only Blank**		0.09
B1, B2	0.1 ng/ml	**Standard**	0.28
C1, C2	0.2 ng/ml	**Standard**	0.40
D1, D2	0.5 ng/ml	**Standard**	0.83
E1, E2	1 ng/ml	**Standard**	1.48
F1, F2	2 ng/ml	**Standard**	2.47
G1, G2	3 ng/ml	**Standard**	2.93
H1, H2	**Positive Control** [Value: 1.0 – 1.8 ng/ml]		1.89
A3, A4	**Sample** [Diluted 1:500]		1.85

Calculated: 500-fold dilution x 1.3 ng/ml = **650 ug/ml**

A typical assay Standard Curve (do not use for calculating sample values)



PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit are mono-specific for ovalbumin, showing no cross-reactivity by immunoelectrophoresis or immunodiffusion with any other chicken egg or serum protein. Pooled sera of the following species showed no reactivity above background in the ELISA assay when tested at 1:100 dilution: rat, human, hamster, goat, bovine (or 10% fetal bovine serum), horse, monkey, dog, chicken, rabbit, and guinea pig.

Precision

Samples containing low and high concentrations of ovalbumin 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 were calculated for the concentrations using a 4-parameter curve-fitting program.

Sample	Ovalbumin ng/ml	Intra-assay % CV	Inter-assay % CV
High Value	2.21	**5.6**	**6.0**
Low Value	0.74	**8.6**	**12.9**

Commercial Vaccine Testing

Chicken embryo allantoic fluid and two commercial vaccines, one prepared from allantoic fluid and the other from chick cell culture, were assay for Ovalbumin levels.

Sample	Preparation	Ovalbumin ng/ml
Allantoic Fluid	Harvested at 9-11 days	**340**
Flu Vaccine (Sanofi Pasteur)	Chicken embryo allantoic fluid	**300**
MMR Vaccine (Merck)	Chick cell culture	**0**

Results: Significant levels of ovalbumin were measured in allantoic fluid and in the Influenza vaccine prepared with viruses grown in and isolated from allantoic fluid. No ovalbumin was detected in the MMR vaccine whose mumps, measles, and rubella viruses were derived from chick cell culture.

Also see:
Waibel KH, Gomez R. 2010. J Allergy Clin Immunol. 125(3): 749-751.
{Ovalbumin content in 2009 to 2010 seasonal and H1N1 monovalent influenza vaccines.}

QUALITY CONTROL

Reagents

Accurate and reproducible assay results rely on proper storage, handling, and control of reagent and sample temperature. Store all reagents as indicated and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls

A Positive Serum Control is provided with the kit, assigned with an Ovalbumin 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 Sample Diluent blank should also be run; OD should be <0.4 and lower than the 0.1 ng/ml Standard OD.

Standard Curve

The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard with a difference greater than 1.2 OD between the lowest and highest Standard. Non-uniform or low signals may indicate problems with technique, protocol directions, and/or reagent preparation, use, or stability. Do not rely on results generated from an assay with these issues.

Technique

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

Equipment

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

Instruction Manual No. M-6050-HS

Chicken Egg Ovalbumin High Sensitivity

ELISA Kit Cat. #6050-HS, 96 Tests

For Quantitation of Egg Ovalbumin in Solution

For research use only, not for diagnostic or therapeutic use.

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ELISA Kit Components	Amount	Part No.
Anti-Ovalbumin Microwell Strip Plate	8-well strips (12)	6051
Ovalbumin Positive Control	0.65 ml	6052
Ovalbumin Standard 0.1 ng/ml	0.65 ml	6053B
Ovalbumin Standard 0.2 ng/ml	0.65 ml	6053C
Ovalbumin Standard 0.5 ng/ml	0.65 ml	6053D
Ovalbumin Standard 1 ng/ml	0.65 ml	6053E
Ovalbumin Standard 2 ng/ml	0.65 ml	6053F
Ovalbumin Standard 3 ng/ml	0.65 ml	6053G
Anti-Ovalbumin Detection Antibody (100X)	0.15 ml	6055
Streptavidin HRP Conjugate (100X)	0.15 ml	S-HRP100
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-6050-HS