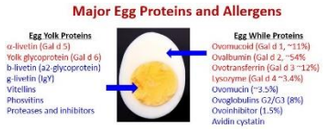
INTENDED USE

The Rat Anti-Ovalbumin IgG ELISA Kit is an indirect ELISA suitable for quantifying or titering IgG class antibodies specific for ovalbumin in serum, plasma or other biological fluids, including tissue culture medium. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

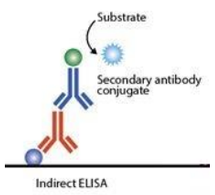
GENERAL INFORMATION



Ovalbumin (Ova) is one of the major allergens in chicken egg white, and is often the cause of hypersensitivity reactions to food. Ova 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 are sufficient to induce severe and rarely fatal hypersensitivity reactions in children with egg allergies.

PRINCIPLE OF THE TEST



The Rat Anti-Ovalbumin IgG ELISA kit is based on the binding of anti- ovalbumin IgG in samples to ovalbumin immobilized on the microwells, and anti- ovalbumin IgG antibody is detected by anti-Rat IgG antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue), which is directly proportional to the amount of antibody present in the sample. Stop Solution is added (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The activity of antibody in samples is calculated relative to supplied calibrators.

PRODUCT SPECIFICATIONS

Assay Specificity

Purified ovalbumin is used to coat the microwells; thus the assay is specific for antibodies directed to ovalbumin. The anti-Rat IgG HRP conjugate reacts with rat IgG antibodies bound to ovalbumin on the plate; IgE, IgM and IgA class antibodies would not be measured above background.

Assay Sensitivity

The ovalbumin coating and anti-Rat IgG/HRP levels are optimized to differentiate anti-ovalbumin IgG from background (non-antibody) signal with rat serum samples diluted 1:100 in the provided diluent. Sensitivity may be increased (with increased background) by using lower sample dilutions (ex. 1:50)

KIT CONTENTS

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

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
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 ambient temperature until kit is used entirely.
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/Conjugate Diluent** and store at 2-8° C until the kit lot expires or is used up.
Anti-Rat IgG - HRP Conjugate Concentrate (100x) Part No. H-RtG.211, 0.15ml	Peroxidase conjugated anti-Rat IgG in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; **10ul** of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Ovalbumin Microwell Strip Plate	6011	8-well strips (12)	Coated with ovalbumin, and post-coated with stabilizers.
Anti-Ovalbumin Calibrators			
50 U/ml 100 U/ml 250 U/ml 500 U/ml	600-140A 600-140B 600-140C 600-140D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-ovalbumin levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Rat Anti-Ovalbumin IgG Positive Control	610-103PC	0.65 ml	Rat anti-ovalbumin IgG. Net OD > **0.5**
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Diluted 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 and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 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.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent.

Assay Design

Review Calculation of Results and Limits of the Assay (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be <0.5 OD. This is usually 1/100 or greater dilution for rat sera with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required (**See Methods A&B**).
- Run a set of **Calibrators**. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve has limitations. See Limits of the Assay (p7).
- Run the Rat Anti-Ovalbumin IgG **Positive Control**; net OD should be >0.5.
- Run a range of sample dilutions for expected higher positives that allows calculation of antibody **Titer** (when specific titer is at least 4-fold higher than non-immune)**. See Method C.**
- Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate.

Plate Set-up

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

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator 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 for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

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 calibrators, 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 – 30 min; 5 washes]

- Add 100ul of diluted Anti-Rat IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. 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).

4. 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.

5. 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.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

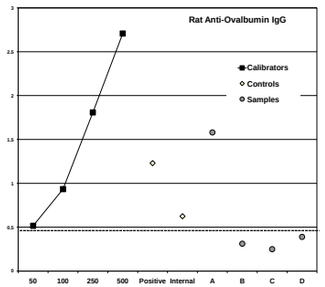
ASSAY RESULTS & PERFORMANCE

Method A. Antibody Activity Threshold Index

Compare Samples to **50 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-ovalbumin IgG, either natural or from immunization, is controlled so that the **50 U/ml Calibrator** represents a threshold OD for most true positives in rat serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of anti-ovalbumin antiserum, derived from ovalbumin immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

50 U/ml: a line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – clearly positive (>0.5 net OD)

Internal Control – a true low positive from an immunized animal that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the 50 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (B, C, D) are negative: below the threshold; 1 sample (A) is positive: clearly above the threshold.

The 50 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- ❖ Divide each Sample net OD by the 50 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

Calibrator Values

The Calibrators are composed of dilutions of anti-ovalbumin antibodies. Values are assigned as arbitrary anti-ovalbumin activity units (see Limits of the Assay, page 7).

ASSAY RESULTS & PERFORMANCE (cont)

Method B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.

2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Example:

Experimental Samples are represented as follows:

C – Calibrator
P – Positive Control
I – Internal Control; lab's threshold positive serum
E – Experimental sample

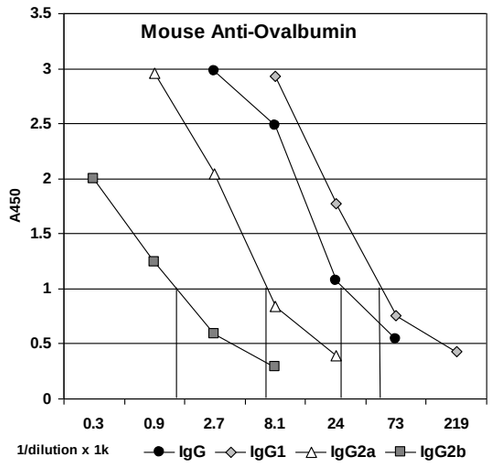
Results

Sample	Assay Net OD		Calculated Antibody Activity	
	Control	Exptl	Control	Exptl
1	0.325	2.281 **C**	0.75	**5.29**
2	0.272	1.581 **C**	0.63	**3.67**
3	0.133	0.998 **C**	0.31	**2.32**
4	0.194	0.453 **C**	0.45	**1.05**
5	0.289	0.767 **E**	0.67	**1.78**
6	0.319	0.982 **P**	0.74	**2.28**
7	0.332	0.491 **I**	0.77	**1.14**
8	0.291	0.351 **E**	0.68	0.81
9	0.402	0.325 **E**	0.93	0.75
10	0.253	0.16 **E**	0.59	0.37
Mean	0.281			
SD	0.075			
Mean +2 SD	**0.431**	= **Positive Index**		

Controls: All are Positive (>1.0) for antibody activity.
Calibrators: Ranking from 50 – 500 U/ml = 1.05 – 5.29.
Experimental: One (1) is Positive (>1.0); 3 are Negative.

ASSAY RESULTS & PERFORMANCE (cont)

Method C. Antibody Titer & Specificity
Dilutions of an antiserum pool from mice hyper-immunized with ovalbumin, using Freund's Adjuvant, were assayed using conjugates specific for the various IgG isotypes. Titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.



Results

Total IgG: Titer: **27.5 k**
IgG1 Subclass: Titer: **56.1 k**
IgG2a Subclass: Titer: **6.9 k**
IgG2b Subclass: Titer: **1.3 k**

The IgG immune response was primarily of the IgG1 subclass.

LIMITATIONS OF THE ASSAY

Quantitation of Antibody in a Sample

The ELISA measures anti-ovalbumin activity, a combination of antibody concentration and avidity for the ovalbumin antigen. Antibodies with similar anti- ovalbumin activities (assay signals) may have substantially different total IgG concentrations, due to differences in avidity. The quantitation or activity of the samples should be appropriately expressed in activity Units (titer), rather than mass units of IgG (e.g., ug/ml).

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in ovalbumin avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the 250 U/ml Calibrator, or another Calibrator in the kit (see Calculation of Results).

Instruction Manual No. M-610-100-OGG

Rat Anti-Ovalbumin IgG ELISA Kit

Cat. No. 610-100-OGG, 96 Tests

For Quantitation of Anti-Ovalbumin IgG in Serum/Plasma or other biological fluids

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

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