INTENDED USE

The Mouse Anti-Ovalbumin IgE ELISA Kit is an immunoassay suitable for quantifying or titering IgE class antibodies specific for ovalbumin in serum, plasma or other biological fluids, including tissue culture medium.

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 Mouse Ovalbumin IgE ELISA kit is based on the binding of anti- ovalbumin IgE in samples to ovalbumin immobilized on the microwells, and anti- ovalbumin IgE antibody is detected by anti-Mouse IgE 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 on the substrate, which is directly proportional to the amount of anti-ovalbumin antibody 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 activity of mouse IgE antibody in samples is calculated relative to Anti-ovalbumin 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-Mouse IgE HRP conjugate reacts with mouse IgE antibodies bound to ovalbumin on the plate; IgG, IgM and IgA class antibodies would not be measured above background.

Assay Sensitivity
The ovalbumin coating and anti-Mouse IgE/HRP levels are optimized to differentiate anti-ovalbumin IgE from background (non-antibody) signal with mouse serum samples diluted 1:25 in the provided diluent.

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).

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-Mouse IgE - HRP Conjugate Concentrate (100x) Part No. H-MsE.211, 0.15ml	Peroxidase conjugated anti-Mouse IgE 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			
100 U/ml 250 U/ml 500 U/ml 1000 U/ml	600-140B 600-140C 600-140D 600-140E	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.
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.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, Positive Control, and Antibody HRP contain bromonitrodioxane (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: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

LIMITS 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 antigens. Antibodies with substantially different total IgE concentrations may display similar anti- ovalbumin activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of IgE (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).

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 (p5-7) and Limits of the Assay (above) 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 1/20 or greater dilution for mouse 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 Method 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, **Method C**, has limitations. See Limits of the Assay (above).
- 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 D.**
- Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

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-Mouse IgE 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.

CALCULATION OF RESULTS

Calculation of Results
Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine **Positive Immune** and **Negative Non-immune**, and to **Quantitate** positive antibody levels.

Method A. Antibody Activity [ELISA Signal & Sample Dilution]

Represent data as net OD units (A450 signal; blank subtracted) ÷ dilution = **Total Activity Units**.

A Calibrator value in the mid-OD range (e.g., 250 U/ml) can be used to normalize inter-assay values.

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 **quantifies** the positive Antibody Activity level.

Example:

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.243	2.358	0.49	**4.79**
2	0.351	0.597	0.71	**1.21**
3	0.286	1.421	0.58	**2.89**
4	0.357	1.268	0.73	**2.58**
5	0.512	0.857	**1.04**	**1.74**
6	0.342	1.296	0.70	**2.63**
7	0.298	0.608	0.61	**1.24**
8	0.285	0.369	0.58	0.75
9	0.157	0.864	0.32	**1.76**
10	0.187	0.543	0.38	**1.10**
Mean	0.302			
SD	0.095			
Mean +2 SD	**0.492**	= **Positive Index**		

CALCULATION OF RESULTS (continued)

Method C. Use of a Calibrator Curve

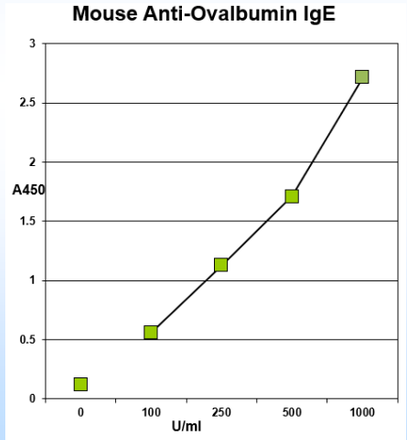
When the dilution curves of samples are parallel to the Calibrator curve (see Limits of the Assay), the anti-Ovalbumin activity units may be determined by interpolation from the Calibrator curve, as follows:

1. The results may be calculated using any immunoassay software package. If software is not available, anti-Ovalbumin activity concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the calibrators (y-axis) against the concentration (U/ml) of anti-Ovalbumin (x-axis). Draw the best fit curve through these points to construct the calibrator curve. A point-to-point construction is most common and reliable.
4. The anti-Ovalbumin activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 1000 U/ml calibrator should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators		A450 nm
A1,2	**Sample Diluent Blank**		0.09
B1,2	100 U/ml	**Calibrator**	0.53
C1,2	250 U/ml	**Calibrator**	1.07
D1,2	500 U/ml	**Calibrator**	1.71
E1,2	1000 U/ml	**Calibrator**	2.78
F1,2	Sample	**1:25**	0.92

Sample Result: **230** U/ml x 25 dilution = **5.75** kU/ml



CALCULATION OF RESULTS (continued)

Method D. Titers from Sample Dilution Curves

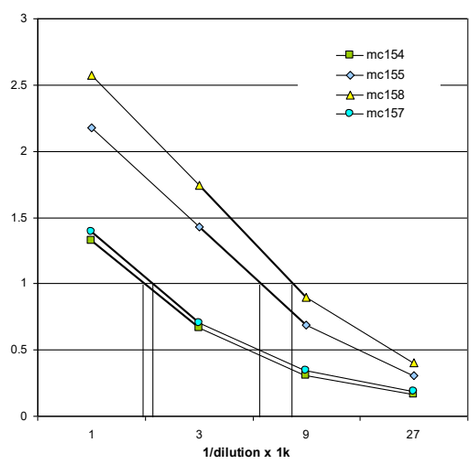
The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. A Calibrator value in the mid-OD range (e.g., 250 U/ml) can be used to normalize inter-assay values.

Calculations
1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index
   = **Total IgE Antibody Activity Units**

Example:

II. A **1.0 OD** Index was used to determine titer of 4 antibodies.



Titer Values
mc154 = 1.72 kU
mc155 = 5.70 kU
mc157 = 1.85 kU
mc158 = 7.90 kU

Instruction Manual No. M-600-165-OGE

Mouse Anti-Ovalbumin IgE

ELISA Kit Cat. No. 600-165-OGE

For Quantitation of Anti-Ovalbumin IgE in Serum

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ELISA Kit Components	Amount	Part
Ovalbumin Coated Microwell Strip Plate	8-well strips 12)	6011
Anti-Ovalbumin Calibrator 100 U/ml	0.65 ml	600-140B
Anti-Ovalbumin Calibrator 250 U/ml	0.65 ml	600-140C
Anti-Ovalbumin Calibrator 500 U/ml	0.65 ml	600-140D
Anti-Ovalbumin Calibrator 1000 U/ml	0.65 ml	600-140C
Anti-Mouse IgE HRP Conjugate (100X)	0.15 ml	H-MsE.211
Sample Diluent (20X)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-600-165-OGE