DATA SHEET

Mouse Anti-Ovalbumin IgG1

ELISA Kit Cat. No. 600-110-OG1

For Semi-Quantitative Determination of
Anti-Ovalbumin IgG1 in Biological Fluids

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INTENDED USE

The Mouse Anti-Ovalbumin IgG1 ELISA Kit is an immunoassay suitable for quantifying or titering IgG1 subclass antibodies specific for ovalbumin in serum, plasma or other biological fluids, including tissue culture medium.

RESEARCH USE OF THE TEST

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 IgG1 ELISA kit is based on the binding of mouse anti-ovalbumin IgG1 in samples to ovalbumin immobilized on the microwells, and anti-ovalbumin IgG1 antibody is detected by anti-mouse IgG1 specific 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 IgG1 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 amount of Mouse IgG1 in samples is calculated relative to mouse anti-ovalbumin reference calibrators.

PRODUCT SPECIFICATIONS

Specificity

Purified ovalbumin is used to coat the microwells; thus the assay is specific for antibodies directed to ovalbumin. The anti-Mouse IgG1 HRP conjugate is specific for mouse IgG1 and does not react with any other IgG subclass, IgM, IgA or IgE.

Assay Sensitivity

The diluted anti-Mouse IgG1 HRP produces a 1.0 OD signal with 20ng of mouse IgG1 coated on a microwell (30 min anti-Mouse IgG1 HRP incubation). The ovalbumin antigen coating level is optimized to differentiate anti-ovalbumin IgG1 from background (non-antibody) signal with mouse serum samples diluted 1:100.

Calibrator Values

The Calibrators are composed of dilutions of ascites from mice immunized with ovalbumin. 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 2-8°C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

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 ambient temperature until kit is used entirely.
Anti-Mouse IgG1 - HRP Conjugate Concentrate (100x) Part No. MsH-G1, 0.15ml	Peroxidase conjugated anti-Mouse IgG1 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.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Ovalbumin Microwell Strip Plate	6011	8-well strips (12)	Coated with ovalbumin, and post-coated with stabilizers.
Mouse Ovalbumin IgG1 Calibrators			
125 U/ml 250 U/ml 500 U/ml 1000 U/ml	600-110-B 600-110-C 600-110-D 600-110-E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing mouse ascites with anti-ovalbumin IgG1 levels in arbitrary Activity Units; diluted in buffer with protein, detergents and ProClin 300 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 pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Mouse IgG1-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.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. 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 Working Sample Diluent. If samples will not be assayed immediately, stored refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

Samples, Calibrators and Controls

Dilute **Samples** in Working Sample Diluent according to expected anti-ovalbumin activity levels; for serum: dilute at least 100-fold (e.g., 10ul sample + 990 ul Diluent) for reduced nonspecific signals. At least 2 dilutions of each sample is recommended in order to determine if reading values from the Calibrator curve is valid (see Limitations of the Assay).

Do not dilute the **Calibrators**. Include Working Sample Diluent as a Negative Control to determine proper assay performance (signal should be < 0.3 OD) and to subtract from sample and Calibrator values to obtain net OD. Internal **Controls** that represent the lab's expected results should also be included in each assay run.

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 10 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 before sample addition to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat 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 - 60min; 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 - 30min; 5 washes]**

- Add 100ul of diluted Anti-Mouse IgG1-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. **Substrate Incubation** **[100ul - 15min]**

- 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

Several data reduction methods may be considered to optimize precision and to best represent the relationships among experimental and control groups.

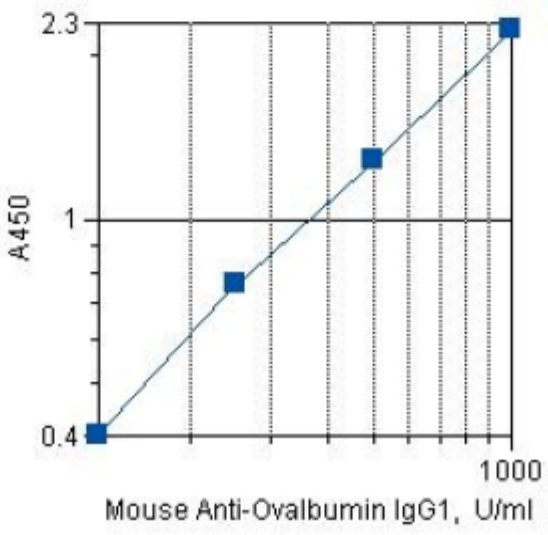
I. Use of a Calibrator Curve

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 & Samples	A450 nm	IgG1 U/ml
A1, A2	**Negative Diluent Control**	0.07	0
B1, B2	125 U/ml **Calibrator**	0.40	125
C1, C2	250 U/ml **Calibrator**	0.76	250
D1, D2	500 U/ml **Calibrator**	1.28	500
E1, E2	1000 U/ml **Calibrator**	2.23	1000
F1, F2	**Sample** [Diluted 1:100]	1.04	350
	Calculated: 100-fold dilution x 350 U/mll = **35.0 kU**/ml in serum		



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CALCULATION OF RESULTS (continued)

II. Antibody Activity Units (Titer)

When the dilution curves of samples are not parallel to the Calibrator curve, antibody potency can be expressed in semi-quantitative activity units, using one of the Calibrators as the Index:

1. Calculate the mean net ODs for replicate samples and the selected Calibrator.
2. Divide each sample OD value by the Calibrator OD value, and multiply by the sample dilution and the Calibrator (U/ml) value = **Total Activity Units**

Typical Results: see Data Table in Section I.

0.97 [Sample, net OD] ÷ **0.69** [250 U/ml Cal, net OD]
x **100** dilution x **250** U/ml = **35.1 kU**/ml Activity Units in serum.

III. Positive Index

Experimental sample values may be expressed relative to the values of Control samples, by calculation of a Positive Index, as follows:

1. Calculate the mean + 2 SD of the Control samples = Positive Index.
2. Divide each sample value by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are negative for antibody.

Typical Results:

	ELISA Units		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		

ELISA Kit Components	Amount	Part No.
Ovalbumin-coated Microwell Strip Plate	8-well strips (12)	6011
Mouse Ovalbumin IgG1 Calibrator 125 U/ml	0.65 ml	600-110-B
Mouse Ovalbumin IgG1 Calibrator 250 U/ml	0.65 ml	600-110-C
Mouse Ovalbumin IgG1 Calibrator 500 U/ml	0.65 ml	600-110-D
Mouse Ovalbumin IgG1 Calibrator 1000 U/ml	0.65 ml	600-110-E
Anti-Mouse IgG1 HRP Conjugate (100X)	0.15 ml	MsH-G1
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	80171
Product Manual	1 ea	600-110-OG1

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Anti-Mouse IgG1-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.

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 substantially different specific IgG1 concentrations may display similar anti-ovalbumin activities, due to differences in avidity. The quantitation or potency of the samples is, therefore, appropriately expressed in activity Units (titer),rather than mass units of IgG1 (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 anti-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).