# Anti-Ovalbumin IgG1 (mouse) ELISA Kit

Item No. 500830

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# GENERAL INFORMATION

## Materials Supplied

| Item Number | Item | 96 wells Quantity/Size |
|---|---|---|
| 400830 | Goat Anti-Mouse IgG1 HRP Detection Antibody | 1 vial/1.5 ml |
| 400832 | Ovalbumin Precoated 96-Well Strip Plate | 1 plate |
| 400834 | Anti-Ovalbumin IgG1 (mouse) ELISA Standard | 1 vial/200 ng |
| 400108 | Immunoassay Buffer D Concentrate (5X) | 4 vials/10 ml |
| 400062 | Wash Buffer Concentrate (400X) | 1 vial/5 ml |
| 400035 | Polysorbate 20 | 1 vial/3 ml |
| 400074 | TMB Substrate Solution | 1 vial/12 ml |
| 10011355 | HRP Stop Solution | 1 vial/12 ml |
| 400012 | 96-Well Cover Sheet | 3 ea |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Anti-Ovalbumin IgG1 (mouse) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab-coat) when using this material.



## If You Have Problems

**Technical Service Contact Information**

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Email:** techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. An orbital microplate shaker
3. Adjustable pipettes and a repeating pipettor.
4. A source of pure water; glass distilled or deionized water is acceptable.
5. Materials used for **Sample Preparation** (see page 9).

# INTRODUCTION

## Background

Immunization of mice with chicken egg albumin (ovalbumin) in a precipitate complex with aluminum hydroxide (alum) is a highly effective means of inducing a potent Th2-mediated immune response.[1-6] Ovalbumin/alum-immunized mice produce anti-ovalbumin antibodies predominantly of the IgG1 and IgE isotypes that mediate tissue-specific effector functions in multiple mouse models of chronic inflammation, including allergic asthma, allergic rhinitis, and cutaneous hypersensitivity.[1-4]

When using one of these models, it is often desirable to measure anti-ovalbumin antibody levels in the plasma or serum to determine the effectiveness of the immunization, the activity of a drug, or the effect of a specific gene deletion. IgG1 is the predominant anti-ovalbumin immunoglobulin isotype found in the serum or plasma of mice immunized with ovalbumin/alum; the plasma concentration of ovalbumin-specific IgG1 is typically 1,000-fold greater than that of ovalbumin-specific IgE.[1] Therefore, the measurement of anti-ovalbumin IgG1 is a commonly used method of assessing the magnitude of this Th2 immune response.

## About This Assay

Cayman's Anti-Ovalbumin IgG1 (mouse) ELISA Kit is an immunometric (*i.e.*, "sandwich") assay that can be used to measure anti-ovalbumin of the IgG1 isotype in mouse plasma and serum without prior sample purification. Affinity-purified anti-ovalbumin IgG1 isolated from the plasma of mice immunized with ovalbumin/alum is used as the standard. The standard curve spans the range of 1.56-100 ng/ml, with a lower limit of quantification (LLOQ) of 1.56 ng/ml.



## Principle Of This Assay

This assay is an indirect ELISA, where each well of the microwell plate supplied in the kit has been coated with ovalbumin. Antibodies specific for ovalbumin, if present in the mouse plasma or serum will bind to the immobilized ovalbumin. A detection antibody conjugated to horseradish peroxidase (HRP), which recognizes mouse IgG1 is added to the well. The excess reagents are washed away and the presence of anti-ovalbumin IgG1 in the sample is determined by measuring the the enzymatic activity of HRP using the chromogenic substrate 3,3'5,5'-tetramethylbenzidine (TMB). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound detection antibody-HRP conjugate, which is proportional to the concentration of the anti-ovalbumin antibodies present in the samples.

**Absorbance $\propto$ [Goat Anti-mouse IgG1/HRP] $\propto$ [Anti-ovalbumin antibody]**

A schematic of this process is shown in Figure 1, on page 8.

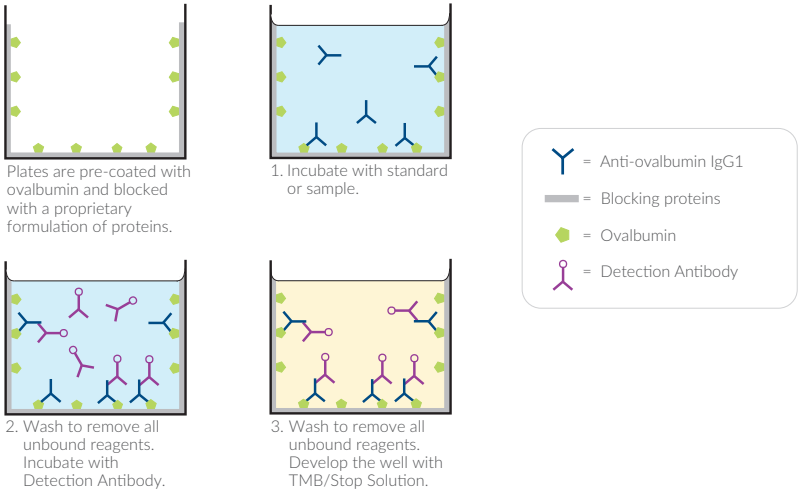



**Figure 1. Schematic of the ELISA**

## Definition of Key Terms

**LLOQ (Lower Limit of Quantification):** the lowest standard concentration in which absorbance (450 nm) - (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.).

**LLOD (Lower Limit of Detection):** the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

**Standard Curve:** a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of analyte.



# PRE-ASSAY PREPARATION

## Buffer Preparation

*Store all diluted buffers at 4°C; they will be stable for about two months.*

1. **Assay Buffer (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D Concentrate (5X) (Item No. 400108) with 40 ml of pure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with pure water.*

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with pure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

## Sample Preparation

Prior to use, it is recommended that serum or plasma samples from ovalbumin/alum-immunized mice be diluted in Assay Buffer (1X) at least 1:2,000 in order to fall within the range of the standard curve (see Table 4 on page 19). In general, mouse serum or plasma (prepared using heparin or EDTA as the anticoagulant) can be used directly in the assay following dilution in Assay Buffer (1X).

## Preparation of Assay-Specific Reagents

### Anti-Ovalbumin IgG1 (mouse) ELISA Standard

Reconstitute the lyophilized Anti-Ovalbumin IgG1 (mouse) ELISA Standard (Item No. 400834) with 2.0 ml of Assay Buffer (1X). Mix gently. The concentration of this solution (the stock standard) is 100 ng/ml. The reconstituted standard is stable for two weeks at 4°C. Enough standard is provided to produce four duplicate-well standard curves for use on different days, if necessary.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes or plastic microfuge tubes and label them #1 through #8. Aliquot 250 µl of Assay Buffer (1X) into tubes #2-8. Transfer 500 µl of freshly prepared stock standard (100 ng/ml) to tube #1. Serially dilute the standard by removing 250 µl from tube #1 and placing into tube #2. Mix gently. Next, remove 250 µl from tube #2 and place into tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any standard to tube #8. This tube is the background control. The diluted standards will be stable for two hours at room temperature.

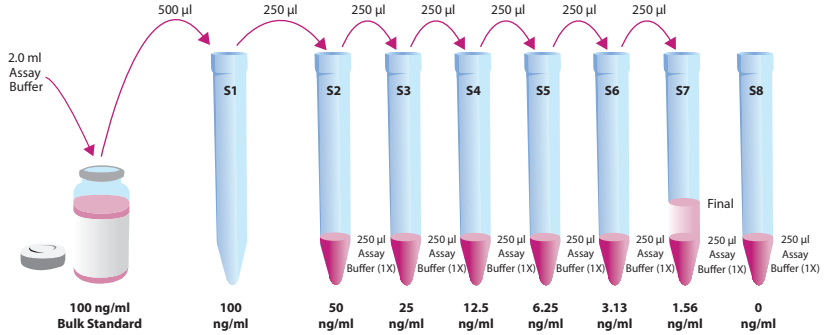



**Figure 2. Preparation of the anti-ovalbumin IgG1 (mouse) standards**



### Goat Anti-Mouse IgG1 HRP Detection Antibody

This reagent is supplied as a concentrated (30X) stock solution of goat anti-mouse IgG1 polyclonal antibody conjugated to HRP. To assay a full plate, dilute 0.4 ml of Goat Anti-Mouse IgG1 HRP Detection Antibody (Item No. 400830) in 11.6 ml Assay Buffer (1X) just prior to use. For a half-plate, dilute 0.2 ml of Goat Anti-Mouse IgG1 HRP Detection Antibody into 5.8 ml Assay Buffer (1X). Any unused diluted detection antibody solution should be discarded.

## Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, assaying samples in triplicate is recommended.

A suggested plate format is shown in Figure 3, below. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested to record the contents of each well on the template sheet provided (see page 22).

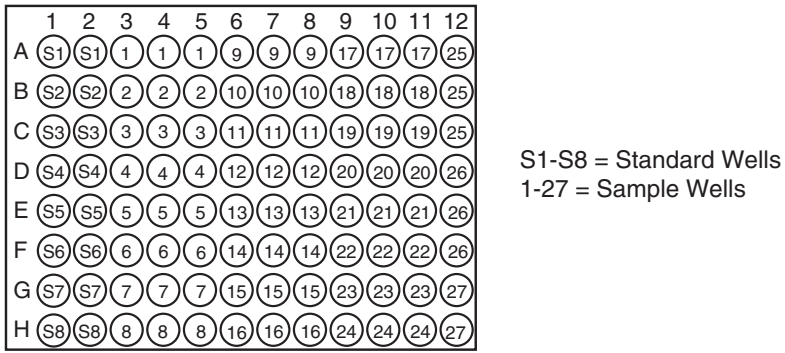



**Figure 3. Sample plate format**



## Performing the Assay

**Pipetting Hints**

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

### Addition of Standards and Samples and First Incubation

1. Add 100 µl of the standards or diluted sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with 96-Well Cover Sheet (Item No. 400012). Incubate for two hours at room temperature on an orbital shaker.

### Addition of Goat Anti-Mouse IgG1 HRP Detection Antibody and Second Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). Each well should be completely filled with wash buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer (1X).
2. Add 100 µl of the diluted detection antibody to each well of the plate.
3. Cover the plate with the 96-Well Cover Sheet and incubate for one hour at room temperature on an orbital shaker.

### Development of the Plate

1. Empty the wells and rinse four times with ~300 µl Wash Buffer (1X).
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the 96-Well Cover Sheet and incubate for 30 minutes at room temperature on an orbital shaker. Development of the blue color can be monitored at 650 nm.
4. DO NOT WASH THE PLATE. After the 30 minute TMB incubation, add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells will remain colorless. Read the plate within 30 minutes after adding the stop solution. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

### Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.



# ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively, a spreadsheet program can be used.

## Calculations

### Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) for standards (S1-S8) *versus* concentration (linear x-axis) and fit the data with a quadratic equation. Using the equation of the line, calculate the concentration of anti-ovalbumin IgG1 in each sample.

## Performance Characteristics

### Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve each time the assay is performed. Do not use the data below to determine the values of your samples.

**Absorbance (450 nm) at 30 minutes**

| Anti-Ovalbumin (mouse) Standard (ng/ml) | Absorbance (450 nm) Blank Adjusted | %CV* Intra-Assay Precision | %CV* Inter-Assay Precision |
|---|---|---|---|
| 100 | 1.87 | 5.4 | 2.9 |
| 50 | 1.07 | 3.1 | 2.2 |
| 25 | 0.581 | 3.9 | 3.9 |
| 12.5 | 0.274 | 3.9 | 8.1 |
| 6.25 | 0.157 | 5.4 | 8.5 |
| 3.13 | 0.08 | 4.6 | 8.6 |
| 1.56 | 0.046 | 6.5 | 14.1 |
| 0 | 0.007 | -- | -- |

**Table 1. Typical results**
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

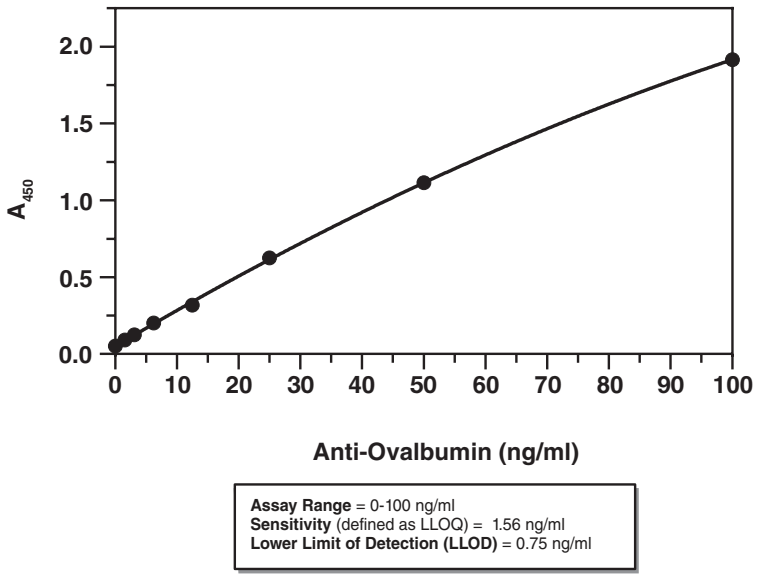



**Figure 4. Typical standard curve**

## Precision

Intra-assay precision was determined by analyzing 24 replicates of three mouse plasma (EDTA) controls in a single assay.

| Matrix Control (µg/ml) | %CV |
|---|---|
| 267.1 | 7.3 |
| 120.6 | 7.7 |
| 24.7 | 5.5 |

**Table 2. Intra-assay precision**

Inter-assay precision was determined by analyzing replicates of three mouse plasma (EDTA) controls in eight separate assays on different days.

| Matrix Control (µg/ml) | %CV |
|---|---|
| 276.3 | 5.8 |
| 131.2 | 5.9 |
| 25.4 | 7.2 |

**Table 3. Inter-assay precision**

| Dilution | Measured Anti-Ovalbumin (µg/ml) | Dilutional Linearity |
|---|---|---|
| Plasma 1 | | |
| 2,000 | 425 | 100% |
| 4,000 | 475 | 112% |
| 8,000 | 469 | 110% |
| 16,000 | 481 | 113% |
| Plasma 2 | | |
| 8,000 | 1,371 | 100% |
| 16,000 | 1,318 | 96% |
| 32,000 | 1,303 | 95% |
| 64,000 | 1,343 | 98% |
| Plasma 3 | | |
| 4,000 | 744 | 100% |
| 8,000 | 734 | 99% |
| 16,000 | 779 | 105% |
| 32,000 | 766 | 103% |
| Plasma 4 | | |
| 8,000 | 1,396 | 100% |
| 16,000 | 1,543 | 111% |
| 32,000 | 1,386 | 99% |
| 64,000 | 1,440 | 103% |

**Table 4. Dilutional linearity of mouse plasma samples.** Plasma from four BALB/c female mice immunized with ovalbumin/alum was tested at multiple dilutions and the concentration of anti-ovalbumin IgG1 was determined using Cayman's Anti-Ovalbumin IgG1 (mouse) ELISA Kit.

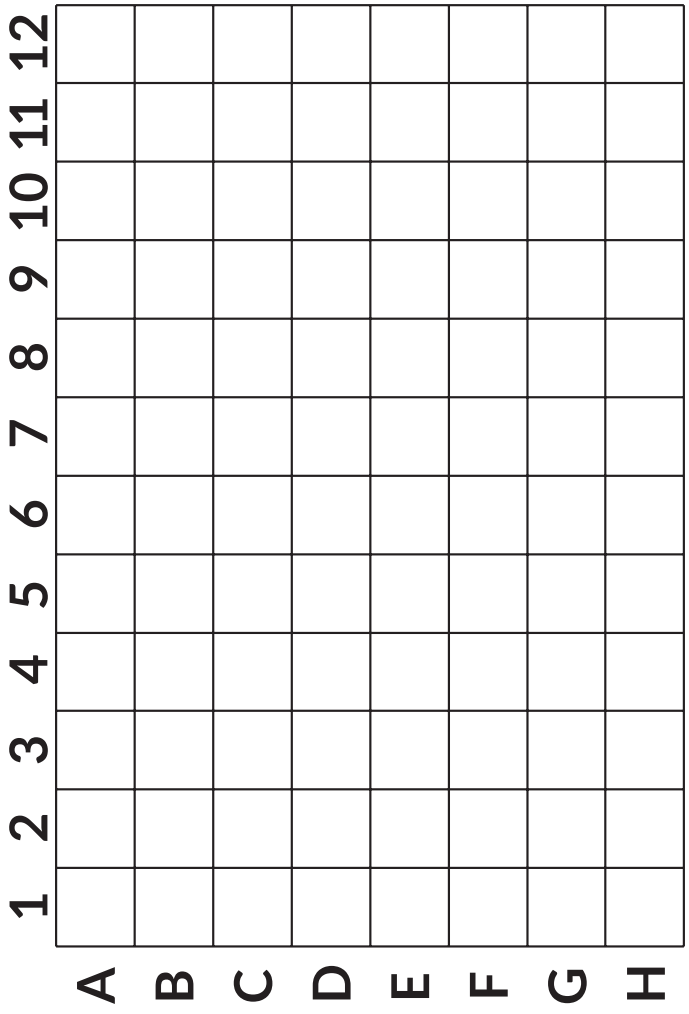
# RESOURCES

## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates | A. Trace organic contaminants in the water source<br>B. Poor pipetting/technique | A. Replace activated carbon filter or change source of pure water |
| Poor development (low signal) of standard curve | A. Plate required more development time<br>B. Standard was diluted incorrectly<br>C. Standard is degraded | |

## References


1. Hogan, S.P., Mould, A., Kikutani, H., *et al. J. Clin. Invest*. **99(6)**, 1329-1339 (1997).
2. Kennedy, J.D., Hatfield, C.A., Fidler, S.F., *et al. Am. J. Respir. Cell Mol. Biol.* **12(6)**, 613-623 (1995).
3. Saito, H., Howie, K., Wattie, J., *et al. Immunology* **104(2)**, 226-234 (2001).
4. Sawada, K., Nagai, H., Basaki, Y., *et al. Clin. Exp. Allergy* **27(2)**, 225-231 (1996).
5. Pichavant, M., Goya, S., Hamelmann, E., *et al. Curr. Protoc. Immunol.* **79**, 15.18.1-15.18.19 (2007).
6. Kung, T.T., Jones, H., Adams, G.K.I., *et al. Int. Arch. Allergy Immunol.* **105(1)**, 83-90 (1994).






## NOTES

## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.