Anti-Ovalbumin IgG1 (mouse) ELISA Kit

Item No. 500830

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Technical Support 888.526.5351
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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
400054	Immunoassay Buffer B Concentrate (10X)	2 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 covers

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

Fax: 734-971-3640

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. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled or deionized water is acceptable.
4. Materials used for **Sample Preparation** (see page 9).

INTRODUCTION

Background

Immunization of mice with chicken egg albumin (ovalbumin/OVA) in a precipitate complex with aluminum hydroxide (alum) is a highly effective means of inducing a potent Th2-mediated immune response.[1-6] OVA/alum immunized mice produce anti-OVA 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-OVA 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-OVA immunoglobulin isotype found in the serum or plasma of mice immunized with OVA/alum; the plasma concentration of OVA-specific IgG1 is typically 1,000-fold greater than that of OVA-specific IgE.[1] Therefore, the measurement of anti-OVA 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 which 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 OVA/alum is used as the standard. The standard curve spans the range of 1.56-200 ng/ml, with an LLOQ of 1.56 ng/ml.

Description of Immunometric ELISAs

Each well of the microwell plate supplied in the kit has been coated with ovalbumin. Antibodies specific for ovalbumin, if present in the biological fluid sample, will bind to the immobilized ovalbumin. A detection antibody recognizing mouse IgG1 is added to the well. This Goat Anti-mouse IgG1 is labeled with HRP, allowing quantitation of the autoantibody. Addition of the HRP Substrate 3,3',5,5'-tetramethylbenzidine (TMB), followed by Stop Solution produces a yellow colored product which can be measured spectrophotometrically. The intensity of the color is directly proportional to the amount of bound Goat Anti-mouse IgG1/HRP, which is proportional to the concentration of the anti-ovalbumin antibody.

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

A schematic of this process is shown in Figure 1, below.

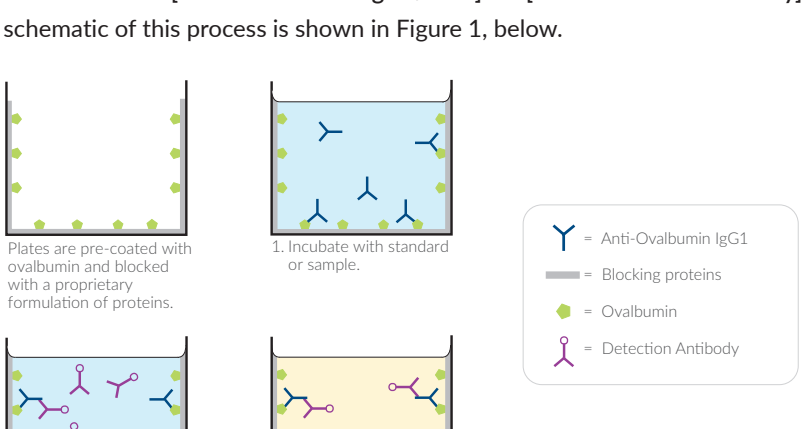


Figure 1. Schematic of the Immunometric ELISA

PRE-ASSAY PREPARATION

Buffer Preparation

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

1. **Assay Buffer Preparation**

   Dilute the contents of one vial of Immunoassay Buffer B Concentrate (10X) (Item No. 400054) with 90 ml of 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 water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer Concentrate (400X) (Item No. 400062): Dilute to a total volume of 2 L with water and add 1 ml of Polysorbate 20 (Item No. 400035).

NOTE: 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 OVA/alum-immunized mice be diluted in Assay Buffer at least 1:2,000 in order to fall within the range of the standard curve (see Table 4 on page 20). 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.

Preparation of Assay-Specific Reagents

Anti-Ovalbumin IgG1 (mouse) ELISA Standard

Reconstitute the lyophilized purified Anti-ovalbumin IgG1 (mouse) ELISA Standard (Item No. 400834) with 1.0 ml of Assay Buffer. Mix gently. The concentration of this solution (the bulk standard) is 200 ng/ml. The reconstituted standard is stable for two weeks at 4°C. Enough standard is provided to produce two 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 into tubes #2-8. Transfer 500 µl of freshly prepared stock standard (200 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 #4-8.

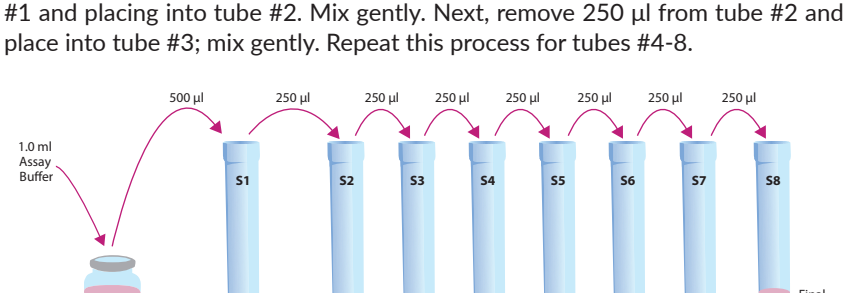


Figure 2. Preparation of the Anti-Ovalbumin IgG1 (mouse) standards

Goat Anti-Mouse IgG1 HRP Detection Antibody

This reagent is supplied as a concentrated (20X) stock solution of Goat anti-mouse IgG1 polyclonal antibody conjugated to HRP. Just before use, prepare a Working Solution by adding 0.6 ml of the Goat Anti-mouse IgG1 HRP Detection Antibody (Item No. 400830) to 11.4 ml Assay Buffer (12 ml total). In the event that two or more experiments are performed with this kit, sufficient stock solution has been provided to produce additional 12 ml of the Working Solution. Discard any leftover Working Solution.

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 according to the plate insert 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, we recommend assaying samples in triplicate.

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. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 15, for more details). We suggest you record the contents of each well on the template sheet provided (see page 22).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	27
H	S8	S8	8	8	8	16	16	16	24	24	24	27

S1-S8 - Standards 1-8
1-27 - Samples

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 four times with Wash Buffer. 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.
2. Add 100 µl of the Detection Antibody working solution to each well of the plate.
3. Cover the plate with plastic film 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 Wash Buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with plastic film 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 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 plate within 30 minutes after adding 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. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

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

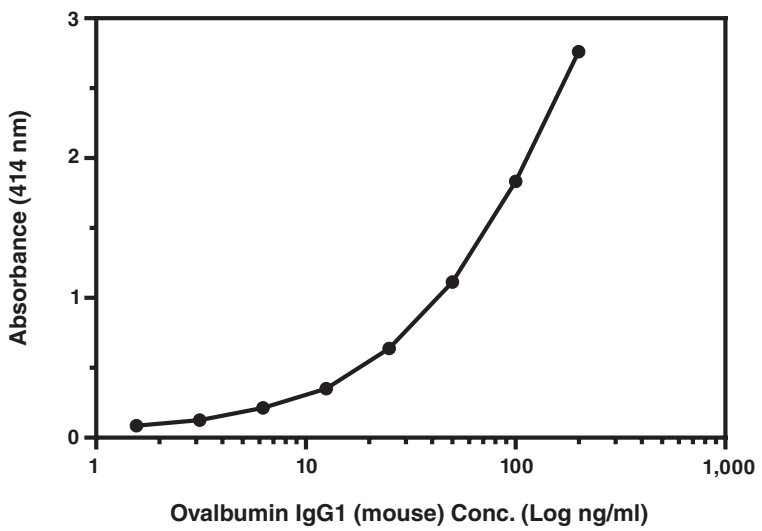
Sample Data

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. Do not use the data below to determine the values of your samples.

30 minute TMB development, absorbance read at 450 nm after addition of Stop Solution.

Anti-OVA IgG_1 (ng/ml)	Absorbance	
200	2.762	2.759
100	1.841	1.825
50	1.117	1.111
25	0.639	0.641
12.5	0.350	0.355
6.25	0.219	0.207
3.12	0.125	0.125
1.56	0.088	0.085

Table 1. Typical results



Assay Range = 1.56-200 ng/ml
LLOQ = 1.56 ng/ml

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. – (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.).The standard was diluted with Assay Buffer.

Figure 4. Typical standard curve

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 17 and in the table below.

Anti-OVA IgG_1 (ng/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
200	3.79	5.72
100	3.65	4.61
50	1.58	5.49
25	2.52	5.15
12.5	4.00	6.42
6.25	5.93	10.03
3.12	20.18	17.76
1.56	31.68†	35.44†

Table 2. Intra- and inter-assay variation

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

†Outside of the recommended usable range of the assay.

Anti-OVA IgG1 (ng/ml)	Mean of O.D.	Standard Deviation (S.D.)	O.D. - (1.64 x S.D.)
200	2.875	0.055	2.784
100	1.788	0.048	1.709
50	1.034	0.014	1.011
25	0.604	0.012	0.584
12.5	0.348	0.010	0.331
6.25	0.210	0.007	0.198
3.12	0.129	0.008	0.114
1.56	0.112	0.008	0.125
0	0.012	0.011	0.030*

*O.D. + (1.64 x S.D.)

Table 3. Determination of LLOQ

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. - (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.). The LLOQ is 1.56 ng/ml.

Dilution Factor	Calculated Anti-OVA IgG1 concentration (mg/ml)				
	Plasma 1	Plasma 2	Plasma 3	Plasma 4	Plasma 5
500	---	---	---	---	---
1,000	---	---	---	---	---
2,000	0.425	---	---	---	---
4,000	0.475	---	1.129	0.744	---
8,000	0.469	1.371	1.058	0.734	1.396
16,000	0.481	1.318	1.079	0.779	1.543
32,000	0.461	1.303	1.147	0.766	1.386
64,000	0.411	1.343	1.121	0.795	1.440
Mean	0.454	1.334	1.107	0.769	1.441
S.D.	0.029	0.030	0.037	0.026	0.072

Table 4. Reproducibility of the assay over a wide dilution range
Plasma from five BALB/c female mice immunized with OVA/alum was diluted from 1:500 to 1:64,000 and the concentration of anti-OVA IgG1 determined using Cayman's Anti-Ovalbumin IgG1 (mouse) ELISA Kit.
--- = outside standard curve range

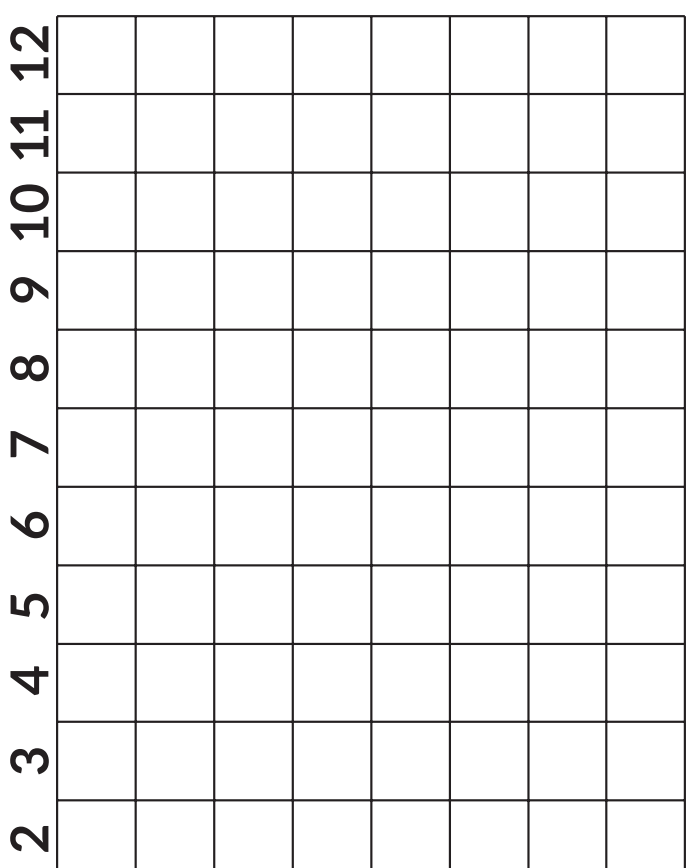
RESOURCES

Troubleshooting

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