

## Mouse Anti-OVA IgG Antibody Assay Kit

Catalog # 3011

*For Research Use Only - Not Human or Therapeutic Use*

### INTRODUCTION

In order to study the pathogenesis of allergic diseases, mice will be the most useful experimental animals, since a variety of inbred strains and transgenic and gene knockout mice are available. Serum IgE level is often raised in allergic diseases and parasitic infections, although serum IgE level alone does not reflect the allergic state and the clinical symptoms. However, it is apparent that a raised level of IgE aids the diagnosis of these diseases in humans.

Since ovalbumin (OVA) is one of the most widely used antigen for studying allergic diseases in mice (4-7), Chondrex provides three types of ELISA kits to determine OVA-specific mouse IgE and IgG antibodies and one kit to determine mouse total serum IgE levels for different purposes. Importantly, the ratio of total IgE and OVA specific IgE in individual specimens can be compared easily and accurately since all these IgE related kits use an identical IgE standard (Clone EC-1).

1. Mouse Anti-OVA IgE Antibody Assay Kit (catalog # 3004)
2. Mouse Total IgE Detection Kit (catalog # 3005)
3. Mouse Serum Anti-OVA IgE Antibody Assay Kit (catalog # 3010)
4. Mouse Serum Anti-OVA IgG Antibody Assay Kit (catalog # 3011)

The cross-linkage of IgE bound to the receptor on mast cells by polyvalent allergen triggers degranulation of mast cells. Although the pathogenic roles of IgG antibodies against the allergen are not well characterized, it is likely that IgG antibodies also may contribute and affect allergic diseases in some aspects. For example: 1) IgG might compete with IgE, 2) IgG might form cross-linkage between allergens bound to IgE on the mast cell, and 3) IgG might create aggregated allergens carrying multivalent IgE epitopes. Chondrex provides this kit (catalog # 3011) for determining anti-ovalbumin (OVA) IgG antibody levels in mouse serum for studying the contribution of IgG in allergic diseases.

### KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgG (Clone L-71 - Dr. Shin Yoshino of Kobe Pharmaceutical University)	1 vial	1000 ng/vial, lyophilized	-20°C
Ovalbumin (OVA)	1 vial	100 µg/vial, lyophilized	-20°C
Detection Antibody (Peroxidase-Conjugated Goat Anti-Mouse IgG Polyclonal Antibody)	2 vials	1 µg/vial, lyophilized	-20°C
Solution A - OVA Dilution Buffer	1 bottle	10 mL	-20°C
Solution B - Blocking Buffer	1 bottle	10 mL	-20°C
Solution C - Sample/Standard/Detection Antibody Dilution Buffer	1 bottle	50 mL	-20°C
TMB Solution	1 bottle	10 mL	4°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 mL	-20°C
Wash Buffer, 20X	1 bottle	50 mL	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

## NOTES BEFORE USING ASSAY

- Note 1: It is recommended that the standard and samples be run in duplicate.
- Note 2: Partially used reagents may be kept at  $-20^{\circ}\text{C}$ .
- Note 3: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, it is necessary to warm the wash buffer by placing the bottle in warm water until crystals have dissolved completely.
- Note 4: Measure exact volume of buffers using a serological pipette prior to diluting. Extra buffer is provided.
- Note 5: Serum IgG antibodies are a mixture of multiple antibodies with a variety of affinity ranges. The OD value obtained in ELISA for antibody assay depends on the concentration of antibody as well as the affinity of antibody with an antigen. In general, ELISA data using a monoclonal antibody as a standard does not reflect the absolute levels of IgG. Therefore, IgG level determined by this kit should be expressed as ng of IgG equivalent to L-71 per mL.

## ASSAY PROCEDURE

- Add OVA Solution:** Dissolve one vial of Ovalbumin (OVA) with 10 mL of OVA Dilution Buffer (Solution A). Add 100  $\mu\text{L}$  of OVA solution to each well and incubate at  $4^{\circ}\text{C}$  overnight.
- Dilute Wash Buffer:** Dilute 50 mL of 20X wash buffer in 950 mL of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- Add Blocking Buffer:** Add 100  $\mu\text{L}$  of the Blocking Buffer (Solution B) to each well and incubate at room temperature for 1 hour.
- Prepare Standard Dilutions:** The recommended standard range is 0.2-12.5 ng/mL. Dissolve one vial of Standard (IgG: 1,000 ng/vial) in 1 mL of Sample/Standard/Detection Antibody Dilution Buffer (Solution C). Take 25  $\mu\text{L}$  of the standard solution and add to 1975  $\mu\text{L}$  of Solution C to make 12.5 ng/mL of IgG solution. Then, serially dilute it with Solution C. For example, mix 250  $\mu\text{L}$  of the standard (25 ng/mL) with an equal volume of Solution C to make 6.25 ng/mL solution, and then repeat it five more times for 3.125, 1.6, 0.8, 0.4 and 0.2 ng/mL standards.
- Prepare Sample Dilutions:** The dilution of serum from mouse immunized with OVA varies from 1:10 or more depending on the immunization schedule and timing of serum collection. In general, no IgG antibody against OVA is determined in normal serum at 1:10 dilution.
- Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- Add Standards and Samples:** Add 100  $\mu\text{L}$  of standards, Solution C (blank) and samples to wells in duplicate. Incubate at room temperature for 2 hours or at  $4^{\circ}\text{C}$  overnight. (OD values may be higher if standards and samples are incubated overnight at  $4^{\circ}\text{C}$ .)
- Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add Detection Antibody:** Dissolve one vial of Detection Antibody in 10 mL Sample/Standard/Detection Antibody Dilution Buffer (Solution C). Add 100  $\mu$ L of detection antibody solution to each well and incubate at room temperature for 1 hour.
10. **Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
11. **Add TMB:** Add 100  $\mu$ L of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.
12. **Stop:** Stop the reaction by adding 50  $\mu$ L of 2N sulfuric acid (Stop Solution) to each well.
13. **Read Plate:** Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

Figure 1 - A typical standard curve for mouse anti-OVA IgG assay

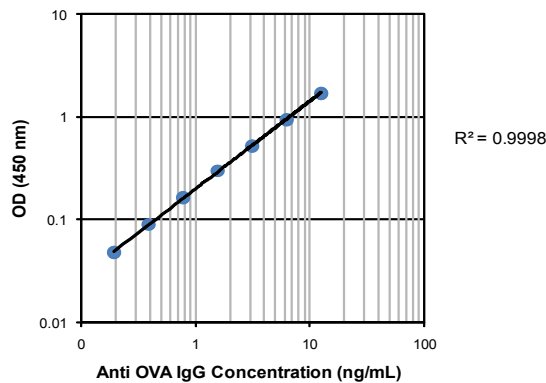


Table 1 - Reproducibility of data assayed by Mouse Anti-OVA IgG Antibody Assay Kit

Test At	2.1 ng/mL	4 ng/mL	8.8 ng/mL
Inter-Assay CV (%)	3.7	0.4	3.3
Intra-Assay CV (%)	9.5	5.2	2.6
Spiking Test*	0.4 ng/mL - 107%	1.6 ng/mL - 95%	6.3 ng/mL - 93%

A pooled normal mouse serum was added with known amounts of IgG, and then diluted with Sample/Standard/Detection Antibody Dilution Buffer for assaying anti-OVA IgG antibody by ELISA.

## REFERENCES

1. Morokawa T. et al. Differential susceptibility of C57BL/6 and DBA/2 mice to ovalbumin-induced pulmonary eosinophil regulated by Th1/Th2 type cytokines. *Immunol. Letter* 70: 127-134 (1999).
2. Oshiba A. et al. Passive transfer of immediate-hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin IgE and IgG1 in mice. *J. Clin. Invest.* 97: 1398-1408 (1996).
3. Hamelmann E. et al. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness-a murine model. *Allergy.* 54: 297-305 (1999).
4. Taube C. et al. Mast cells, FcεRI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J. Immunol.* 172: 6398-6406 (2004).