

## Mouse Anti-OVA IgE Antibody Assay Kit

Catalog # 3004

*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 (1-4), 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.

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)

Mouse Anti-OVA IgE Antibody Assay Kit (catalog # 3004) is designed to detect anti-OVA IgE antibody for limited samples such as hybridoma cell culture supernatant and known IgE solution for biological tests, which do not contain other classes of antibodies. The detection antibody (rat monoclonal antibody, Clone 345-2) used in this kit reacts equally with both IgE<sup>a</sup> (Balb/c) and IgE<sup>b</sup> (C57BL/6) allotypes, and it is not necessary to run two separate assays using two independent IgE<sup>a</sup> and IgE<sup>b</sup> standards. Clone 345-2 does not crossreact with any mouse immunoglobulin subclasses (IgG1, G2a, G2b, G3 and IgM).

Note: This kit, which employs an OVA-coated plate to detect IgE, cannot be used for assaying IgE in mouse sera due to the competitive binding of other classes of antibodies to the same antigenic epitopes on OVA, since IgE level in serum is only 1:1000 that of IgG level. For assaying IgE anti-OVA antibodies in mouse sera, use Mouse Serum Anti-OVA IgE Antibody Assay Kit (# 3010). Please refer to the protocol of individual assay kits, and select the most suitable one for experimental purposes or contact us for more information.

### KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgE (Clone E-C1 - Dr. Shin Yoshino of Kobe Pharmaceutical University)	1 vial	1000 ng/vial, lyophilized	-20°C
Detection Antibody (Biotinylated Rat Anti-Mouse IgE Clone 345-2 - Kowa Company, Ltd., Tokyo)	1 vial	1 µg/vial, lyophilized	-20°C
Ovalbumin (OVA)	1 vial	100 µL, 1 mg/mL	-20°C
Solution A - OVA Dilution Buffer	1 bottle	10 mL	-20°C
Solution B - Sample/Standard Dilution Buffer	1 bottle	50 mL	-20°C
Solution C - Detection Antibody Dilution Buffer	1 bottle	10 mL	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer	1 bottle	20 mL	-20°C
Streptavidin Peroxidase	2 vials	50 µL	-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)	4°C or -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 IgE antibodies are a mixture of multiple antibodies with wide range of affinity. For example, the affinity of individual monoclonal antibodies with antigen varies significantly among clones and differs by tens to hundreds of times. The OD value obtained in ELISA for antibody assay depends on the concentration of antibody as well as the affinity of antibody with antigen. Therefore, in general, ELISA data using a monoclonal antibody as a standard does not reflect the absolute levels of IgE. Therefore, IgE level determined by this kit should be expressed as ng of IgE equivalent to E-C1 per mL.

## ASSAY PROCEDURE

- Coat Plate with OVA:** Dilute one vial of OVA in 10 mL of OVA Dilution Buffer (Solution A). Add 100  $\mu\text{L}$  of the 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.*
- Prepare Standard Dilutions:** Dissolve one vial of Standard (IgE: 1,000 ng/vial) in 1 mL of Sample/Standard Dilution Buffer (Solution B). Take 50  $\mu\text{L}$  of the standard solution and add to 950  $\mu\text{L}$  of Solution B to make 50 ng/mL of IgE solution. Then, serially dilute it with Solution B. For example, mix 300  $\mu\text{L}$  of the standard (50 ng/mL) with an equal volume of Solution A to make 25 ng/mL solution, and then repeat it five more times for a range of 0.8-50 ng/mL.
- Prepare Sample Dilutions:** Dilute samples with Solution B to the standard range of 0.8-50 ng/mL. It is recommended to assay in duplicate, 2-3 different dilutions when the IgE concentration in the sample is unknown. For example, 1:10, 1:100 and 1:1,000 could be used.
- Add Standards and Samples:** Add 100  $\mu\text{L}$  of standards, Solution B (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.*
- Add Detection Antibody:** Dissolve one vial of detection antibody in 10 mL Detection Antibody Dilution Buffer (Solution C). Add 100  $\mu\text{L}$  of detection antibody solution to each well and incubate at room temperature for 1 hour.

8. **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 Streptavidin Peroxidase:** Dilute one vial of Streptavidin Peroxidase in 10 mL of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100  $\mu$ L of streptavidin peroxidase 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 with 50  $\mu$ L of 2N sulfuric acid (Stop Solution).
13. **Read Plate:** Read the OD values at 450 nm (if possible, use dual beam at 450/630 nm).

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

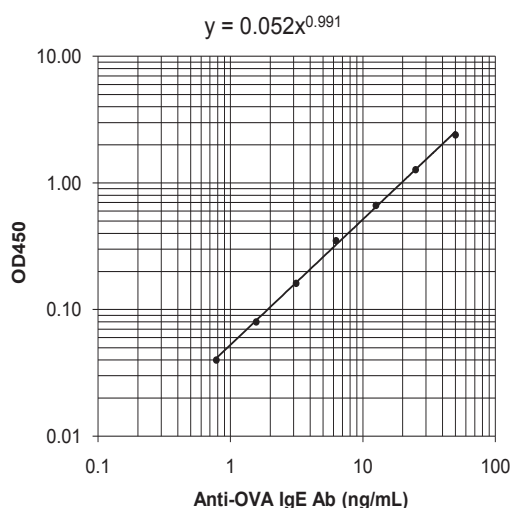


Table 1 - Reproducibility of data assayed by Mouse Anti-OVA IgE Assay Kit

Test At	12.5 ng/mL	25 ng/mL	50 ng/mL
Inter-Assay CV (%)	2.1	2.6	1.7
Intra-Assay CV (%)	1.9	1.6	1.8
Spiking Test*	98%	89%	96%

A pooled normal mouse serum was added with known amounts of IgE, and then diluted with Sample/Standard Dilution Buffer for assaying IgE anti-OVA antibody in 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 Review (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)