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 are the most practical experimental animals, due to the variety of inbred strains and transgenic and gene knockout mice that are available. Serum IgE levels are often raised in allergic diseases and parasitic infections, although serum IgE level alone does not reflect the allergic state and the clinical symptoms of the patient. However, it is apparent that a raised serum IgE level aids in the diagnosis of these diseases in humans. As ovalbumin (OVA) is one of the most widely used antigens for studying allergic diseases in mice (4-7), Chondrex, Inc. provides ELISA kits to determine OVA-specific mouse IgE, IgA, IgM, IgG, and IgG subtype antibodies and to determine mouse total serum IgE and IgA levels.

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 Anti-OVA IgG and IgG subtype Antibody Assay Kit (Catalog # 3011, 3013, 3015, 3016, 3029)
5. Mouse Anti-OVA IgM Antibody Assay Kit (Catalog # 3017)
6. Mouse Anti-OVA IgA Antibody Assay Kit (Catalog # 3018)
7. Mouse Total IgA Detection Kit (Catalog # 3019)

The Mouse Anti-OVA IgE Antibody Assay Kit (Catalog # 3004) is designed to detect anti-OVA IgE antibodies in limited samples such as hybridoma cell culture supernatant and solutions containing anti-OVA IgE antibodies without other subclasses of anti-OVA antibodies present (Note 1). The detection antibody (rat monoclonal antibody, Clone 345-2) used in this kit reacts equally with both IgE^a (Balb/c) and IgE^b (C57BL/6) allotypes, so it is not necessary to run two separate assays using two independent IgE^a and IgE^b standards. Clone 345-2 does not crossreact with any other mouse immunoglobulin subclasses (IgA, IgG, or IgM).

Note 1: This kit employs an indirect ELISA using an OVA-coated plate to detect IgE; this kit does not work for assaying anti-OVA IgE antibodies in mouse sera. The competitive binding of other subclasses of antibodies recognizing the same antigenic epitopes on OVA may reduce anti-OVA IgE binding to the OVA, as the serum IgE level is 1/1000 lower than that of IgG. For assaying anti-OVA IgE antibodies in mouse sera, use the Mouse Serum Anti-OVA IgE Antibody Assay Kit (Catalog # 3010). For more information, please contact us at support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgE (Clone E-C1 - Dr. Shin Yoshino of Kobe Pharmaceutical University) (300412)	1 vial	1000 ng/vial, lyophilized	-20°C
Detection Antibody (Biotinylated Rat Anti-Mouse IgE Clone 345-2 - Kowa Company, Ltd., Tokyo) (300413)	1 vial	Lyophilized	-20°C
Ovalbumin (OVA) (300411)	1 vial	100 µl, 1 mg/ml	-20°C
Solution A - OVA Dilution Buffer (30054)	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (30055)	1 bottle	50 ml	-20°C
Solution C - Detection Antibody Dilution Buffer (30056)	1 bottle	10 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB Solution (contains DMSO) (90023)	2 vials	0.2 ml	4°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

2607 151st Place NE
Redmond, WA 98052
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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°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.

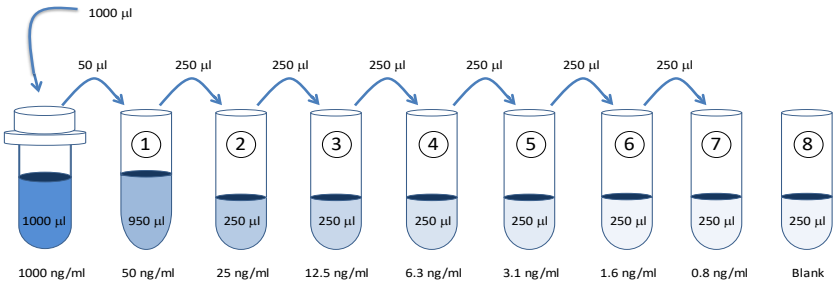
Note 5: Serum IgE antibodies are a mixture of multiple antibodies with wide range of affinity. For example, the affinity of individual monoclonal antibodies with antigen varies significantly among clones and differs by tens to hundreds of times. The OD value obtained in ELISA for antibody assay depends 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

1. **Coat Plate with OVA**: Dilute one vial of OVA in 10 ml of OVA Dilution Buffer (Solution A). Add 100 µl of the OVA solution to each well and incubate at 4°C overnight.

2. **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.*

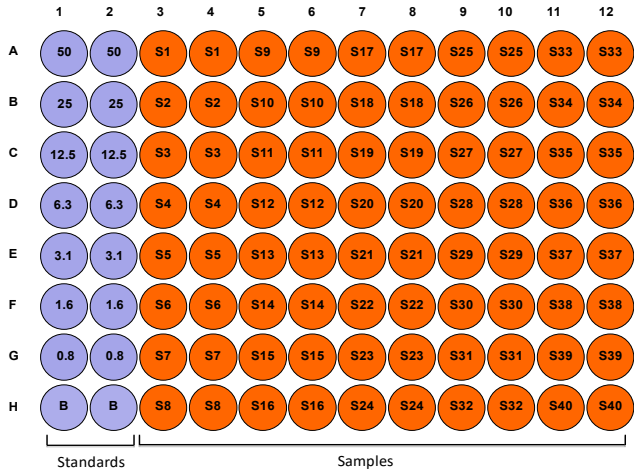
3. **Prepare Standard Dilutions**: Dissolve one vial of Standard (IgE: 1,000 ng/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution B). Take 50 µl of the standard solution and add to 950 µl of Solution B to make a 50 ng/ml IgE standard solution. Then, serially dilute it with Solution B. For example, mix 250 µl of the standard solution (50 ng/ml) with an equal volume of Solution B to make a 25 ng/ml solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, and 0.8 ng/ml standard solutions. The remaining 1000 ng/ml standard stock can be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



4. **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 anti-OVA IgE concentration in the sample is unknown. For example, 1:10, 1:100 and 1:1,000 could be used.

5. **Add Standards and Samples**: Add 100 µl of standards, Solution B (blank), and samples to appropriate wells in duplicate (Figure 1). Incubate at room temperature for 2 hours

Figure 1 - A Standard Assay Layout



6. **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.*

7. **Add Detection Antibody**: Dissolve one vial of detection antibody in 10 ml Detection Antibody Dilution Buffer (Solution C). Add 100 µl 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 µl 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**: Use new tubes when preparing TMB. Dilute one vial of TMB in 10 ml Chromogen Dilution Buffer just prior to use. Add 100 µl 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 µl of 2N sulfuric acid (Stop Solution).

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.

2607 151st Place NE
Redmond, WA 98052
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

CALCULATION OF ANTIBODY CONCENTRATION

1. Average the duplicate OD values for the standards, blanks (B), and test samples.
2. Subtract the averaged blank (B) values from the averaged OD values of the standards and test samples.
3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 2 shows an example of a standard curve for IgE antibodies.
4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration (ng/ml) in original sample specimens.

Figure 2 - A typical standard curve for the mouse anti-OVA IgE assay

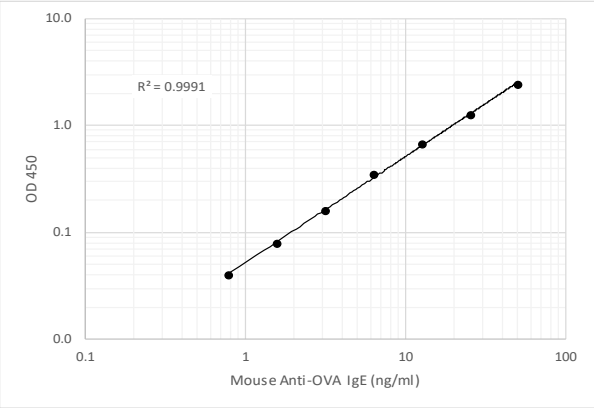


Table 1 - Reproducibility of data assayed by Mouse Anti-OVA IgE Assay Kit

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 to known amounts of IgE, and then diluted with Sample/Standard Dilution Buffer for assaying anti-OVA IgE antibodies 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 marine 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).

2607 151st Place NE
Redmond, WA 98052
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com