Mouse Serum Anti-OVA IgE Antibody Assay Kit

Catalog # 3010

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)

In general, mouse serum contains various types of antibodies against an antigen, such as IgA, IgM and IgG at higher levels than IgE, thus it is difficult to detect IgE antibody levels due to the competition of antigenic determinant on the antigen by other types of antibodies. This kit (catalog # 3010) is designed to detect OVA specific IgE antibody in mouse sera, which contain a mixture of various types of anti-OVA antibodies, and can measure anti-OVA IgE levels accurately in samples with less than 500 ng/ml IgE. This kit consists of two components: an anti-IgE monoclonal antibody to capture IgE in serum samples, and a biotinylated-OVA for determining OVA-specific IgE captured by anti-IgE antibody coated on plate surfaces. The capture antibody (rat anti-mouse IgE antibody, Clone 77-1) used in this kit reacts equally with both IgE^a (Balb/c) and IgE^b (C57BL/6) allotypes, and it is not necessary to run two separate assays using two independent IgE^a and IgE^b standards. Clone 77-1 does not cross-react with any mouse immunoglobulin subclasses (IgG1, IgG2a, IgG2b, IgG3 and IgM).

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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgE (Clone E-C1 - Dr. Shin Yoshino of Kobe Pharmaceutical University)	1 vial	1000 ng, lyophilized	-20°C
Capture Antibody (Rat Anti-Mouse IgE Clone 77-1 - Kowa Company, Ltd., Tokyo)	1 vial	500 μg, lyophilized	-20°C
Biotinylated Ovalbumin	1 vial	10 μg, lyophilized	-20°C
Solution A - Capture Antibody Dilution Buffer	1 bottle	10 ml	-20°C
Solution B - Blocking Buffer	1 bottle	10 ml	-20°C
Solution C - Sample/Standard Dilution Buffer	1 bottle	50 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 (contains DMSO)	2 vials	0.2 ml	-20°C
Chromagen Dilution Buffer	1 bottle	20 ml	-20°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	-20°C

NOTES BEFORE USING ASSAY

1. It is recommended that the standard and samples be run in duplicate.

2. Partially used reagents may be kept at –20°C.

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.

4. Measure exact volume of buffers using a serological pipette prior to diluting. Extra buffer is provided.

5. Serum IgE 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 IgE. Therefore, IgE level determined by this kit should be expressed as ng of IgE equivalent to E-C1 per ml.

6. If the total IgE concentration in a sample is higher than 500 ng/ml, the sample must be diluted to adjust the total IgE levels lower than 500 ng/ml, because the anti-OVA IgE value obtained by this ELISA is lower than the actual values due to competition with non-anti-OVA IgE antibodies in samples (Figure 2). Therefore, it is strongly recommended that total IgE levels be determined first using the Mouse Total IgE Assay Kit (catalog # 3005).

ASSAY PROCEDURE

1. **Add Capture Antibody**: Dilute one vial of Capture Antibody with 10 ml of Capture Antibody Dilution Buffer (Solution A). Add 100 μl of capture antibody 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.*

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3. **Add Blocking Buffer**: Add 100 µl of the Blocking Buffer (Solution B) to each well and incubate at room temperature for 1 hour.

4. **Prepare Standard Dilutions**: The recommended standard range is 0.4-25 ng/ml. Dissolve one vial of Standard (IgE: 1,000 ng/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution C). Take 50 µl of the standard solution and add to 1950 µl of Solution C to make 25 ng/ml of IgE solution. Then, serially dilute it with Solution C. For example, mix 250 µl of the standard (25 ng/ml) with an equal volume of Solution C to make 12.5 ng/ml solution, and then repeat it five more times for 6.25, 3.125, 1.6, 0.8 and 0.4 ng/ml standards.

5. **Prepare Sample Dilutions**: The suggested dilution of serum from mouse immunized with OVA varies from 1:10 to 1:100 depending on the immunization schedule and timing of serum collection. In general, no IgE antibody against OVA was determined in normal serum at 1:10 dilution.

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 Standards and Samples**: Add 100 µl of standards, Solution C (blank) and samples to wells in duplicate. Incubate at room temperature for 90 minutes or at 4°C overnight. (OD values may be higher if standards and samples are incubated overnight at 4°C.)

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 Biotinylated Ovalbumin**: Dissolve one vial of Biotinylated Ovalbumin in 10 ml Sample/Standard Dilution Buffer (Solution C). Add 100 µl of biotinylated ovalbumin solution to each well and incubate at room temperature for 90 minutes.

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 Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

12. **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.*

13. **Add TMB**: Dilute one vial of TMB with 10 ml Chromagen Dilution Buffer just prior to use. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 30 minutes at room temperature.

14. **Stop**: Stop the reaction by adding 50 µl of 2N sulfuric acid (Stop Solution) to each well.

15. **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.

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

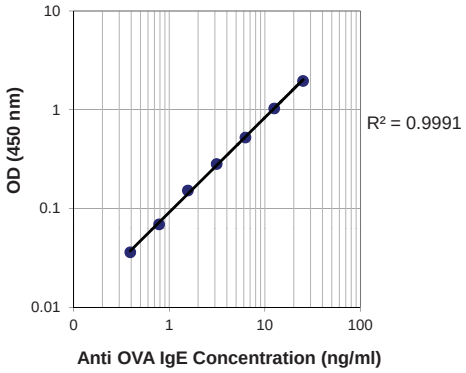
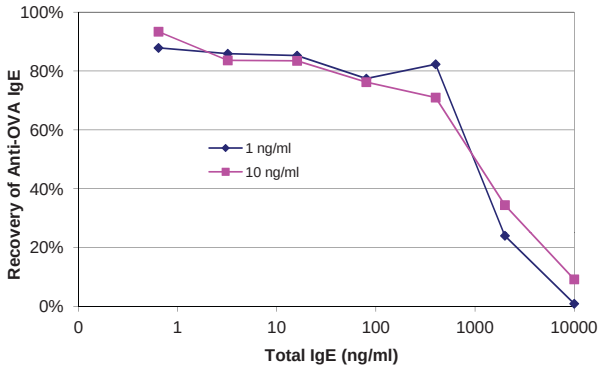


Table 1 - Reproducibility of data assayed by Mouse Serum Anti-OVA IgE Antibody Assay Kit

Test At	2.5 ng/ml	5 ng/ml	10 ng/ml
Inter-Assay CV (%)	4.5	5.3	2.8
Intra-Assay CV (%)	7.1	7.1	7.1
Spiking Test*	82%	89%	92%

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

Figure 2 - Influence of non-anti-OVA IgE antibodies in samples



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