

Mouse Total IgE (IgE^a and IgE^b) Detection Kit

Catalog # 3005

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Type I hypersensitivity, which is characterized by an allergic reaction immediately following contact of innocuous antigens, is a typical clinical feature of allergic diseases, such as asthma, eczema, hay fever and urticaria. This hypersensitivity is mediated by IgE, so called “atopic reagin”, and the clinical features of type I hypersensitivity is described as “atopy”. IgE binds to high affinity IgE receptor (FcεRI) on mast cells and basophils, and drastically up-regulates FcεRI expression through stabilization and accumulation of FcεRI (1) and contributes to enhancing hypersensitivity responses to allergens. The specific allergen bound to IgE on the cell surfaces forms cross-linkage of FcεRI (2-3), which leads to the stimulation and degranulation of mast cells. This is associated with the release of a variety of proinflammatory mediators and cytokines, such as histamine, proteolytic enzymes, heparin, and chemotactic factors, which cause the symptoms associated with type I hypersensitivity.

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)

Mouse Total IgE Detection Kit (catalog # 3005) is designed to determine total IgE levels in mouse sera. 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, and it is not necessary to run two separate assays using two independent IgE^a and IgE^b standards. Clone 345-2 does not cross-react with any mouse immunoglobulin subclasses (IgG1, IgG2a, IgG2b, IgG3 and IgM).

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
Capture Antibody (Clone 77-1 - Kowa Company, Ltd., Tokyo)	1 vial	0.1 mL	-20°C
Detection Antibody (Biotinylated Clone 345-2 - Kowa Company, Ltd., Tokyo)	1 vial	1 µg/vial, lyophilized	-20°C
Solution A - Capture Antibody 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/vial	-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°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: Total IgE levels in normal mouse sera is assumed to be in a range of 50-100 ng/ml, whereas it will increase to several µg/mL at 2 weeks after immunization with an antigen with aluminum hydroxy gel. Total IgE levels will further increase to 10-20 µg/mL after repeating challenge with the aerosolized antigen.

ASSAY PROCEDURE

- 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.
- Prepare Standard Dilutions:** The recommended standard range is 1.6-100 ng/mL. Dissolve one vial of Standard (IgE: 1,000 ng/vial) in 1 mL of Sample/Standard Dilution Buffer (Solution B). Take 100 µL of the standard solution and add to 900 µL of Solution B to make 100 ng/mL of IgE solution. Then, serially dilute it with Solution B. For example, mix 250 µL of the standard (100 ng/mL) with an equal volume of Solution B to make 50 ng/mL solution, and then repeat it five more times for 25, 12.5, 6.25, 3.125 and 1.6 ng/mL standards.
- Prepare Sample Dilutions:** The suggested dilution of normal serum is 1:10 - 1:50, whereas serum from mouse immunized with antigens varies from 1:100 to 1:1000 depending upon the immunization schedule and timing of serum collection.

4. **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.*
5. **Add Standards and Samples:** Add 100 μ L of standards, Solution B (blank) and samples to wells in duplicate. Incubate at room temperature for 2 hours or at 4°C overnight. (OD values may be higher if standards and samples are incubated overnight at 4°C.)
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 μ 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 Dilution Buffer (Solution D). Add 100 μ 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 μ L of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.
12. **Stop:** Add 50 μ 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 total IgE assay

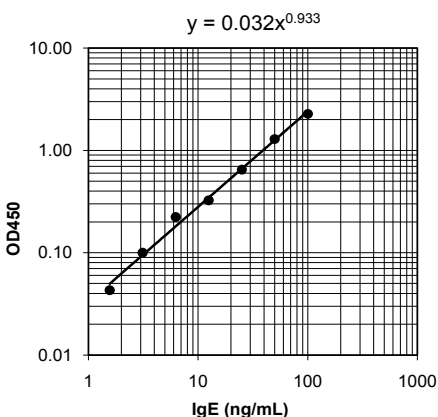


Table 1 - Reproducibility of data assayed by Mouse Total IgE Detection Kit

Test At	12.5 ng/mL	50 ng/mL	100 ng/mL
Inter-Assay CV (%)	3.0	2.1	1.9
Intra-Assay CV (%)	0.3	2.0	3.0
Spiking Test*	122%	109%	100%

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

REFERENCES

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