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 with innocuous antigens, is a typical clinical feature of allergic diseases, such as asthma, eczema, hay fever, and urticaria. This hypersensitivity is mediated by IgE, the so-called "atopic reagin", and the clinical features of type I hypersensitivity are described as "atopy". IgE binds to high affinity IgE receptors (FcεRI) on mast cells and basophils, and drastically up-regulates FcεRI expression through stabilization and accumulation of FcεRI (1), enhancing hypersensitivity responses to allergens. The specific allergen bound to IgE on the cell surfaces cross-links 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 cytokine 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 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 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, so 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 and subtypes (IgA, IgM, IgG, IgG1, IgG2a, IgG2b, IgG2c, and IgG3).

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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgE (30051)	1 vial	100 ng/vial, lyophilized	-20°C
Capture Antibody (Clone 77-1 provided by Kowa Company, Ltd., Tokyo) (30052)	1 vial	0.1 ml	-20°C
Detection Antibody (Biotinylated Clone 345-2 provided by Kowa Company, Ltd., Tokyo) (30053)	1 vial	Lyophilized	-20°C
Solution A - Capture Antibody 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/vial	-20°C
TMB Solution (contains DMSO) (90023)	2 vials	0.2 ml	-20°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

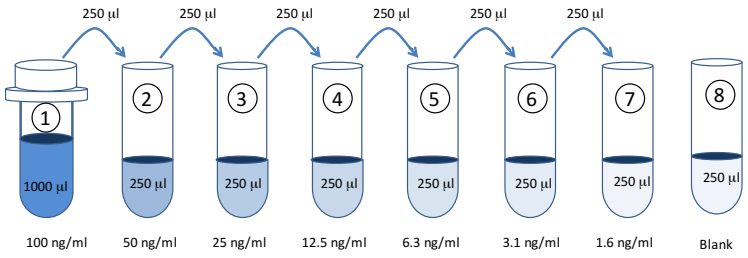
NOTES BEFORE USING ASSAY

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. Total IgE levels in normal mouse sera is assumed to be in the 50-100 ng/ml range, whereas it will increase to several µg/ml 2 weeks after immunizing with an antigen in aluminum hydroxy gel adjuvant. Total IgE levels will further increase to 10-20 µg/ml after repeat challenges with the aerosolized antigen.

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. **Prepare Standard Dilutions**: The recommended standard range is 1.6-100 ng/ml. Dissolve one vial of Standard IgE (100 ng/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution B) to make a 100 ng/ml IgE standard stock 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 a 50 ng/ml solution, and then repeat it five more times for 25, 12.5, 6.25, 3.12, and 1.6 ng/ml standards. The remaining 100 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.



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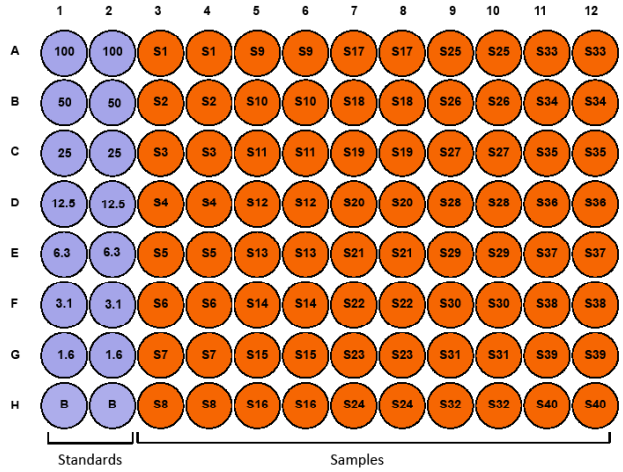
3. **Prepare Sample Dilutions**: The suggested dilution for normal serum is 1:10 - 1:50, whereas serum from mouse immunized with antigens varies from 1:100 to 1:1000 depending upon the immunization schedule and timing of serum collection.

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 µl of standards, Solution B (blank) and samples to appropriate wells (Figure 1) in duplicate. 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 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 with 10 ml of 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**: Add 50 µl 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.

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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 mouse total IgE assay

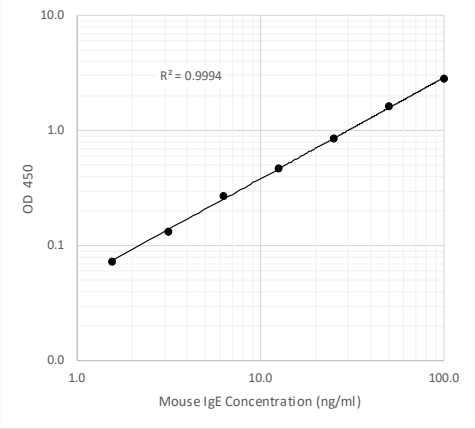

Table 1 - Reproducibility of data assayed by Mouse Total IgE Detection Kit

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
Test At	**3.1 ng/ml**	**12.5 ng/ml**	**50 ng/ml**
Spiking Test*	96%	95%	98%

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

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