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

Catalog # 3005

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify total mouse IgE antibodies
FORMAT:	96-well ELISA plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	100 ng/ml to 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:100 or greater
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (0.3-3%)/Inter-Assay (1.9-3%)/Spiking Test (95-98%)
NOTES:	

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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 antibodies, the so-called "atopic reagin" and the clinical features of type I hypersensitivity are described as "atopy". IgE antibodies bind to high affinity IgE receptors (FcεRI) on mast cells and basophils and drastically up-regulate FcεRI expression through stabilization and accumulation of FcεRI (1), enhancing hypersensitivity responses to allergens. The specific allergen bound to IgE antibodies on the cell surface 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 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 are the most practical experimental animals, due to the variety of inbred strains and transgenic and gene knockout mice that are available. Serum IgE antibody levels are often elevated 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 an elevated serum IgE level aids in the diagnosis of these diseases in humans.

The Mouse Total IgE Detection ELISA Kit (Catalog # 3005) is designed to determine total IgE levels in mouse sera. Both capture and detection monoclonal antibodies used in this kit do not cross-react with any other mouse immunoglobulin subclasses and subtypes (IgA, IgM, IgG, IgG1, IgG2a, IgG2b, IgG2c, and IgG3) and both react equally with both IgEa (Balb/c) and IgEb (C57BL/6) allotypes, so it is not necessary to run two separate assays using two independent IgEa and IgEb standards.

KIT COMPONENTS

Item	Quantity	Amount	Storage
IgE Standard (30051)	1 vial	100 ng/vial, lyophilized	-20°C
Capture Antibody (30052)	1 vial	0.1 ml	-20°C
Detection Antibody (30053)	1 vial	Lyophilized	-20°C
Solution A - Coating Buffer (9052)	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 (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

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ASSAY OUTLINE

Add 100 µl of diluted Capture Antibody to all wells

Incubate at 4 degrees Celsius overnight.
Wash plate.

Add 100 µl of diluted Standards and Samples

Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Detection Antibody solution

Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase solution

Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of TMB solution

Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

Read plates at 450 nm/630 nm

PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	50	50	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	25	25	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	12.5	12.5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	6.3	6.3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	3.1	3.1	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	1.6	1.6	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards | Samples

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NOTES BEFORE USING ASSAY

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

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

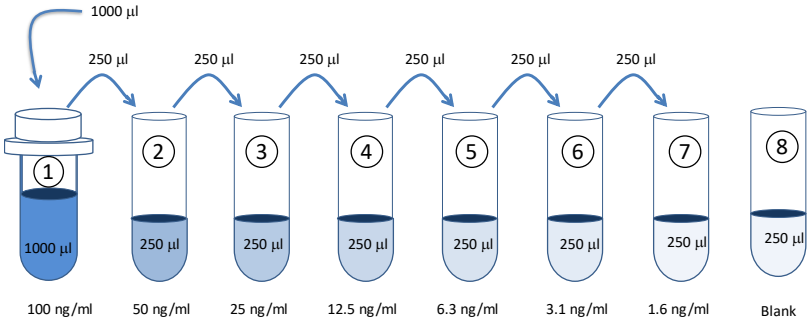
NOTE 8: Total IgE levels in normal mouse sera is assumed to be in the 50-100 ng/ml range, whereas it will increase to several µg/ml 2 weeks after immunizing with an antigen in aluminum hydroxide gel adjuvant. Total IgE levels will further increase to 10-20 µg/ml after repeat challenges with the aerosolized antigen.

ASSAY PROCEDURE

1. **Add Capture Antibody**: Dilute one vial of Capture Antibody with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight. Any leftover Capture Antibody Stock Solution may be stored at -20°C for future assays.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **Prepare Standard Dilutions**: The recommended standard range is 1.6-100 ng/ml. Dissolve one vial of Standard (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, 6.3, 3.1, 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. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



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 blotting 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 in duplicate. Incubate at room temperature for 2 hours.

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 blotting 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). Alternatively, dissolve one vial of Detection Antibody in 50 µl of Solution C and follow the dilutions in the following table. Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution C (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

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 blotting 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.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

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 blotting 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.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

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.

CALCULATING RESULTS

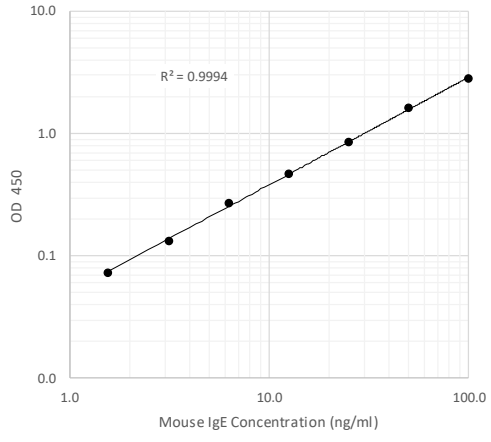
1. Average the duplicate OD values for the blank (B), standards, and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of standards against the concentration of standard antibody (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 1.6 - 100 ng/ml.

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 the original test samples.

Figure 1 - A Typical Standard Curve for the Mouse Total IgE Detection ELISA Kit



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ASSAY VALIDATION

Table 1 - Reproducibility Data for the Mouse Total IgE Detection ELISA Kit

Test	12.5 ng/ml	50 ng/ml	100 ng/ml
Intra-Assay CV (%)	0.3	2.0	3.0
Inter-Assay CV (%)	3.0	2.1	1.9
Test	**3.1 ng/ml**	**12.5 ng/ml**	**50 ng/ml**
Spike Test* (%)	96%	95%	98%

* Known amounts of mouse IgE antibodies were added to samples and then diluted with Sample/Standard Dilution Buffer (Solution B).

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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