Mouse Anti-OVA Serum IgE Antibody Detection ELISA Kit

Catalog # 3010

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-ovalbumin serum IgE antibodies
FORMAT:	96-well ELISA Plate with removeable strips
TYPE:	Sandwich ELISA
ASSAY TIME:	5.5 hours
STANDARD RANGE:	25 - 0.4 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:10 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (7.1%) /Inter-Assay (12.8-5.3%) /Spiking Test (82-92%)
NOTES:	

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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 subtype antibodies and to determine mouse total serum antibody levels. For more information, please visit www.chondrex.com.

In general, mouse serum contains various subtypes of antibodies against an antigen, such as IgA, IgM, and IgG, all at higher levels than IgE. Thus, it is difficult to detect IgE antibody levels in serum due to the competition for the antigenic determinant on the antigen by the other subtypes of antibodies. This kit (Catalog # 3010) is designed to detect OVA specific IgE antibodies in mouse serum and can measure anti-OVA IgE levels accurately in samples with less than 500 ng/ml of 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 antibodies captured by the anti-IgE monoclonal antibody coating the wells. The capture antibody used in this kit reacts 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. It does not cross-react with any mouse immunoglobulin subclasses and subtypes (IgA, IgM, IgG, IgG1, IgG2a, IgG2b, IgG2c, and IgG3).

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgE (30101)	1 vial	1000 ng, lyophilized	-20°C
Capture Antibody (30102)	1 vial	500 µg, lyophilized	-20°C
Biotinylated Ovalbumin (30103)	1 vial	10 µg, lyophilized	-20°C
Solution A - Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution B - Blocking Buffer (30105)	1 bottle	10 ml	-20°C
Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (30106)	1 bottle	50 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	-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	-20°C

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ASSAY OUTLINE

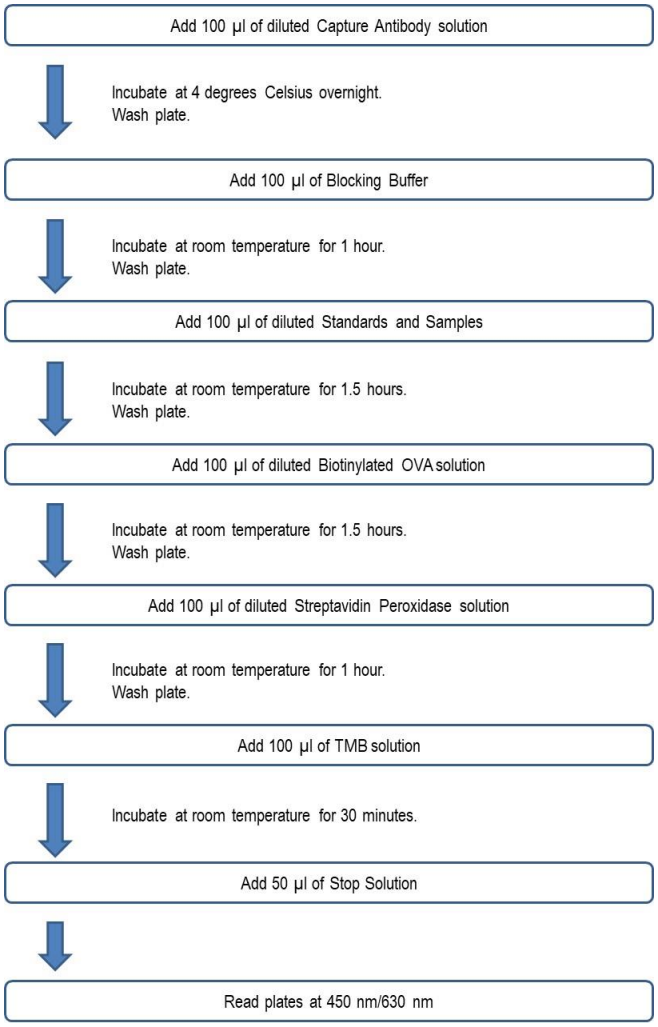
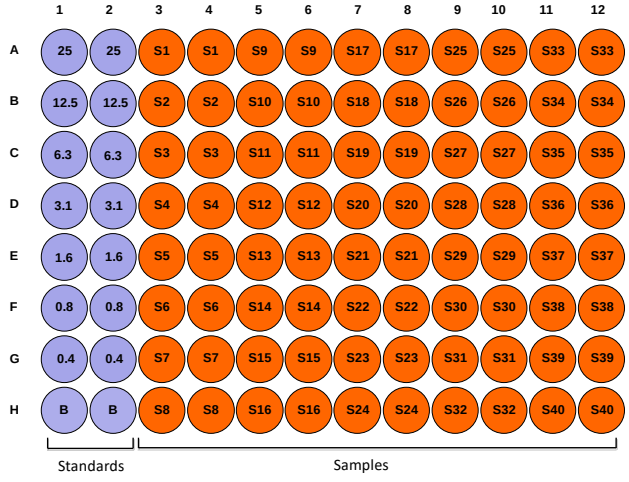


PLATE MAPPING



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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: 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 below 500 ng/ml because the anti-OVA IgE value obtained by this ELISA is lower than the actual value 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 Detection ELISA Kit (Catalog # 3005)

ASSAY PROCEDURE

1. **Add Capture Antibody Solution**: 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. **Wash**: 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.*

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 in 1 ml of Sample/Standard/Secondary Antibody Dilution Buffer (Solution C) and keep it as a 1000 ng/ml standard stock. Take 50 µl of the standard solution (1000 ng/ml) and add it to 1950 µl of Solution C to make a 25 ng/ml IgE standard solution. Then serially dilute it with Solution C. For example, mix 250 µl of the stock solution with an equal volume of Solution C to make a 12.5 ng/ml stock solution, and then repeat it five more times for 6.3, 3.1, 1.6, 0.8, and 0.4 ng/ml standard solutions. The remaining 1000 ng/ml standard stock solution can be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.

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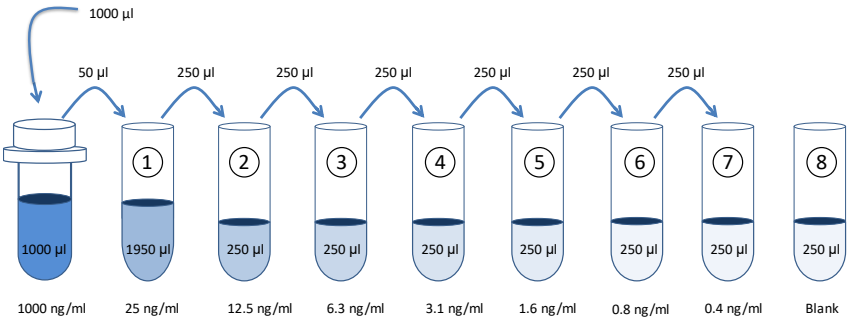

5. **Prepare Sample Dilutions**: The dilution of serum from mouse immunized with OVA varies (1:10 or more) depending on the immunization schedule and timing of serum collection. In general, no IgE antibodies against OVA are observed in normal serum at a 1:10 dilution. Chondrex, Inc. recommends assaying 2-3 different dilutions when the anti-OVA serum IgE concentration in the sample is unknown.

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 Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate and incubate at room temperature for 90 minutes.

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 Biotinylated OVA**: Dissolve one vial of Biotinylated OVA in 10 ml Sample/Standard/Secondary Antibody Dilution Buffer (Solution C). Alternatively, dissolve one vial of Biotinylated OVA in 100 µl of Solution C and dilute according to the following table. Add 100 µl of biotinylated OVA solution to each well and incubate at room temperature for 90 minutes.

Strip #	Biotinylated OVA (µl)	Solution C (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

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 Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 ml Streptavidin Peroxidase 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

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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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

13. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 30 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

14. **Stop**: Stop the reaction with 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.

CALCULATING RESULTS

1. Average the duplicate OD values for the standards, blanks (B), 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 the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve for anti-OVA Serum 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 test samples.

Figure 1 - A Typical Standard Curve for the Anti-OVA Serum IgE Antibody Detection ELISA Kit

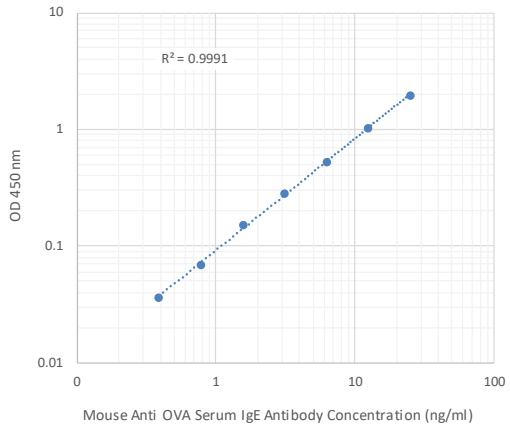

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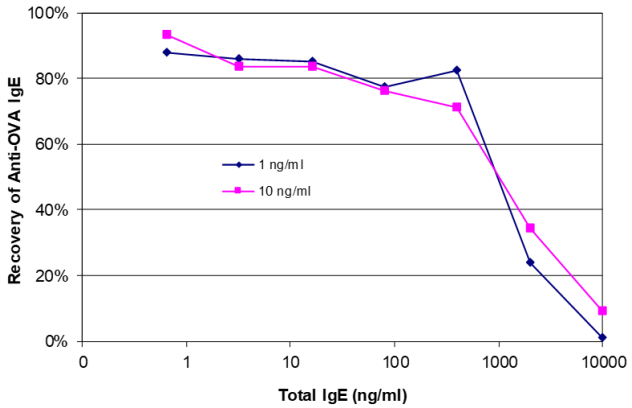
Figure 2 - Influence of Non-Anti-OVA IgE Antibodies in Samples



VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-OVA Serum IgE Antibody Detection ELISA Kit

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

*Known amounts of anti-OVA Serum IgE antibodies were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer (Solution C) to assay anti-OVA Serum IgE antibodies by ELISA.

TROUBLESHOOTING

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

REFERENCES

1. T. Morokawa, *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. A. Oshiba, *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. E. Hamelmann, *et al*. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness-a marine model. *Allergy* **54**:297-305 (1999).

4. C. Taube, *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).

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