Mouse Anti-OVA IgE Antibody ELISA Kit

Catalog # 3004

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse non-serum anti-ovalbumin IgE antibodies
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	50 - 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Culture Media & Purified IgE
RECOMMENDED SAMPLE DILUTIONS:	1:10 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (1.6-1.9%) /Inter-Assay (1.7-2.6%) /Spiking Test (89-98%)
NOTES:	This kit cannot be used to assay mouse anti-OVA IgE antibodies in serum

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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.

The Mouse Anti-OVA IgE Antibody ELISA Kit (Catalog # 3004) is designed to detect anti-OVA IgE antibodies in limited samples such as hybridoma cell culture supernatant and solutions containing anti-OVA IgE antibodies without other subclasses of anti-OVA antibodies present. The secondary 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 other mouse immunoglobulin subclasses or subtypes (IgA, IgM, IgG, IgG1, IgG2a, IgG2b, IgG2c, or IgG3).

Note: This kit is an indirect ELISA using an OVA-coated plate to detect IgE; this kit cannot assay anti-OVA IgE antibodies in mouse serum. The competitive binding of other antibody subclasses recognizing the same antigenic epitopes on OVA may reduce anti-OVA IgE binding to the OVA, as serum IgE levels are usually 1/1000 lower than that of IgG. For assaying anti-OVA IgE antibodies in mouse serum, please use the Mouse Serum Anti-OVA IgE Antibody Detection ELISA Kit (Catalog # 3010). Please contact us at support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Ovalbumin (OVA) (300411)	1 vial	100 µl, 1 mg/ml	-20°C
Standard Mouse IgE (300412)	1 vial	1000 ng, lyophilized	-20°C
Secondary Antibody (300413)	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 - Secondary 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	-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

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ASSAY OUTLINE

Add 100 µl of diluted OVA into wells

↓ Incubate at 4 degrees Celsius overnight.
Wash plate.

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted secondary antibody solution into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted streptavidin peroxidase solution into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of TMB solution into wells

↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution into wells

↓

Read plates at 450 nm/630 nm

PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	50	50	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	25	25	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	12.5	12.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	6.3	6.3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	3.1	3.1	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	1.6	1.6	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.8	0.8	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.

ASSAY PROCEDURE

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

Strip #	OVA (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 0.8-50 ng/ml. Dissolve one vial of Standard in 1 ml of Sample/Standard Dilution Buffer (Solution B) and keep it as a 1000 ng/ml standard stock. Take 50 µl of the standard solution (1000 ng/ml) and add it to 950 µl of Solution B to make a 50 ng/ml IgE standard solution. Then serially dilute it with Solution B. For example, mix 250 µl of the stock solution with an equal volume of Solution B to make a 25 ng/ml stock solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, 0.8 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. recommend making fresh serial dilutions for each assay.

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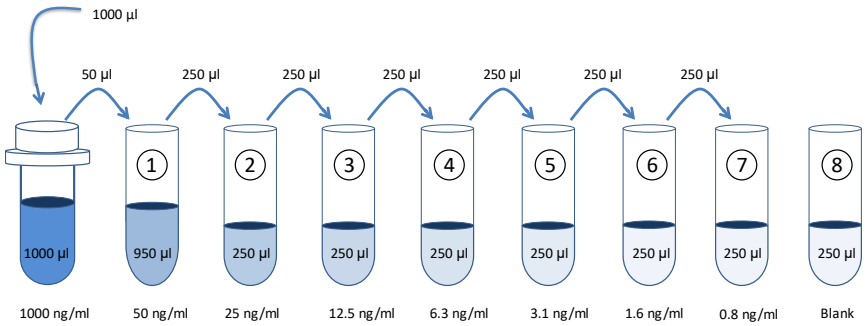

3. **Prepare Sample Dilutions**: The sample dilutions (1:10 or more) depend on the types of samples. Chondrex, Inc. recommends assaying 2-3 different dilutions when the anti-OVA IgE concentration in the sample is unknown.

4. **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.*

5. **Add Standards and Samples**: Add 100 µl of standards, Solution B (blank), and samples to 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 Secondary Antibody**: Dissolve one vial of Secondary Antibody in 10 ml Secondary Antibody Dilution Buffer (Solution C). Alternatively, dissolve one vial of Secondary Antibody in 50 µl of Solution C and dilute according to the following table. Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Secondary 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 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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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 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 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**: Stop the reaction with 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

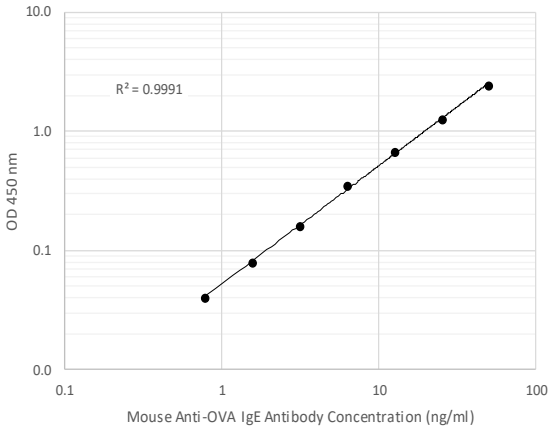
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 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 IgE Antibody ELISA Kit



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VALIDATION DATA

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

Test	12.5 ng/ml	25 ng/ml	50 ng/ml
Intra-Assay CV (%)	1.9	1.6	1.8
Inter-Assay CV (%)	2.1	2.6	1.7
Spike Test* (%)	98%	89%	96%

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

TROUBLESHOOTING

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

REFERENCES

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