Mouse Anti-OVA Antibody Subtype/Subclass ELISA Kits

Catalog # 3011, 3013, 3015, 3016, 3017, 3018, and 3029

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-ovalbumin antibodies
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	3011 (IgG) : 12.5 - 0.2 ng/ml 3013 (IgG1) : 12.5 - 0.2 ng/ml 3015 (IgG2a) : 50 - 0.8 ng/ml 3016 (IgG2b) : 50 - 0.8 ng/ml 3017 (IgM) : 100 - 1.6 ng/ml 3018 (IgA) : 12.5 - 0.2 ng/ml 3029 (IgG2c) : 50 - 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3011: Intra-Assay (2.6-9.5%)/Inter-Assay (0.4-3.7%)/Spiking Test (93-107%) 3013: Intra-Assay (4.7-9.1%)/Inter-Assay (3.6-9.5 %)/Spiking Test (96-114%) 3015: Intra-Assay (5.9-8.9%)/Inter-Assay (6.3-9.4%)/Spiking Test (98-104%) 3016: Intra-Assay (3.9-9.9%)/Inter-Assay (6.3-9.4%)/Spiking Test (94-106%) 3017: Intra-Assay (2.9-4.6%)/Inter-Assay (6.6-9.1%)/Spiking Test (93-116%) 3018: Intra-Assay (0.2-7.8%)/Inter-Assay (1.2-7.9%)/Spiking Test (93-98%) 3029: Intra-Assay (5.5-9.6%)/Inter-Assay (2.9-7%)/Spiking Test (100-109%)
NOTES:	N/A

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INTRODUCTION

Ovalbumin (OVA) is a widely used antigen for inducing allergic reactions in experimental animals (1-4). To study the contribution of antibodies to allergic reactions, Chondrex, Inc. provides a variety of subtype and subclass anti-OVA mouse antibody ELISA kits. Investigators studying the pathogenesis of allergic diseases in mice should consider several issues such as mucosal immunity, antibody responses to different subtypes (IgA, IgE, IgG, and IgM) and subclasses (IgG1, IgG2a, IgG2b, and IgG2c), and even the types of adjuvants used for immunization.

The following may be informative for studies on allergic reactions in experimental animals.

1) Mucosal immunity: The mucosal immune system is the first line of defense against potential pathogenic and non-pathogenic environmental factors such as bacteria, viruses, and dietary proteins. Importantly, poor mucosal immune function may lead to abnormal absorption of mimic antigens such as food components and bacterial cell walls, which elicit antibodies that may cross-react with autologous components, also known as “autoantibodies”. IgG, the dominant immunoglobulin subtype in serum, protects the host from pathogens and unwanted antigens that have penetrated the body. On the other hand, IgA, a known mucosal immunoglobulin, prevents the penetration of pathogens and other unwanted antigenic substances through mucosal membranes. Therefore, to study mucosal and systemic immune responses to allergens in mouse models, OVA is a valuable and convenient antigen (5).

2) Allergy: In general, an allergic reaction is mediated by IgE-antigen complexes. More specifically, IgE molecules cross-linked by a polyvalent antigen on the surfaces of mast cells trigger their degranulation which initiates the ensuing allergic cascade. Although the role of IgG antibodies in allergic reactions is not yet clear, two opposing roles are postulated: 1) IgG antibodies which share epitopes with IgE antibodies may competitively bind the epitopes on the allergen and modulate the allergic reaction, or 2) IgG antibodies may enhance the allergic reaction by providing aggregated allergens to IgE on mast cells. In addition, the roles of antibody subtypes and subclasses may differ depending on the allergic reaction, as IgG1 and IgE are regulated by Th2 cells, whereas IgG2a, IgG2b and IgG2c are dependent on Th1 cells. Thus, to investigate the immune responses involved in allergic reactions in OVA-induced allergic mouse models (1-4), anti-OVA IgE, IgG, and IgA antibody ELISA kits are valuable tools.

3) Adjuvants: The type of adjuvant used can elicit specific antibody subtypes. For example, aluminum adjuvant is widely used to elicit IgE antibodies (7), whereas Cholera toxins are effective at eliciting IgA antibodies (6). Moreover, Complete Freund’s Adjuvant (CFA) is widely used for stimulating IgG and IgM antibody production (8).

LIST OF MOUSE ANTI-OVA ANTIBODY SUBTYPE/SUBCLASS ELISA KITS

Kit	Catalog #
Mouse Anti-OVA IgG Antibody ELISA Kit	3011
Mouse Anti-OVA IgG1 Antibody ELISA Kit	3013
Mouse Anti-OVA IgG2a Antibody ELISA Kit	3015
Mouse Anti-OVA IgG2b Antibody ELISA Kit	3016
Mouse Anti-OVA IgM Antibody ELISA Kit	3017
Mouse Anti-OVA IgA Antibody ELISA Kit	3018
Mouse Anti-OVA IgG2c Antibody ELISA Kit	3029

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KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard	IgG (30111) IgG1 (30131) IgG2a (30151) IgG2b (30161) IgM (30171) IgA (30181) IgG2c (30291)	1 vial	1000 ng, Lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies)	IgG (30113) IgG1 (30133) IgG2a (30153) IgG2b (30163) IgM (30173) IgA (30183) IgG2c (30293)	2 vials	50 µl	-20°C
Ovalbumin (OVA) (300411)		1 vial	100 µl	-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
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

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 -20°C 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: Depending on the isotypes, subtypes, and targeting epitopes of antibodies, the binding affinity of individual antibodies varies significantly. Therefore, the total IgG antibody concentration calculated as the sum of individual IgG subtypes might not perfectly match the total IgG concentration as determined by the Mouse Anti-OVA IgG Antibody ELISA Kit (Cat # 3011).

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ASSAY OUTLINE

Add 100 µl of diluted OVA solution into wells

↓ Incubate at 4 degrees Celsius overnight.
Wash plate.

Add 100 µl of blocking buffer into wells

↓ Incubate at room temperature for 1 hour.
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 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

Example of the Mouse Anti-OVA IgG Antibody ELISA Kit

	1	2	3	4	5	6	7	8	9	10	11	12
A	12.5	12.5	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	6.3	6.3	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	3.1	3.1	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	1.6	1.6	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	0.8	0.8	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	0.4	0.4	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.2	0.2	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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ASSAY PROCEDURE

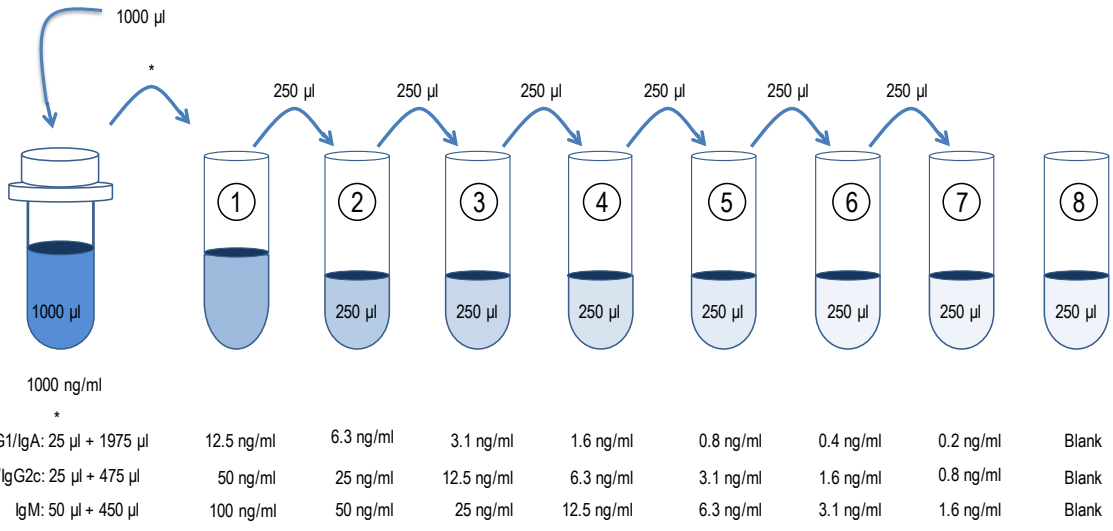
1. **Add OVA Solution**: Dilute one vial of Ovalbumin (OVA) with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 100 µl of OVA solution to each well and incubate at 4°C overnight.

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. **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**: Please see the figure below for each assay's recommended standard range. Dissolve one vial of Standard in 1 ml of Sample/Standard/Secondary Antibody Dilution Buffer (Solution C) and keep it as a standard stock. Dilute the standard stock accordingly to get the first stock solution. Then serially dilute it with Solution C. For example, mix 250 µl of the first stock solution with an equal volume of Solution C to make the second stock solution, and then repeat it five more times. The original standard stock solution may be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



5. **Prepare Sample Dilutions**: The dilution of serum from mouse immunized with OVA varies (1:100 or more) depending on the immunization schedule and timing of serum collection. In general, no antibodies against OVA are observed in normal serum at a 1:100 dilution.

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. Incubate at room temperature for 2 hours.

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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 Secondary Antibody**: Dilute one vial of Secondary Antibody in 10 ml Sample/Standard/Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	2nd 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

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 IgG 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.

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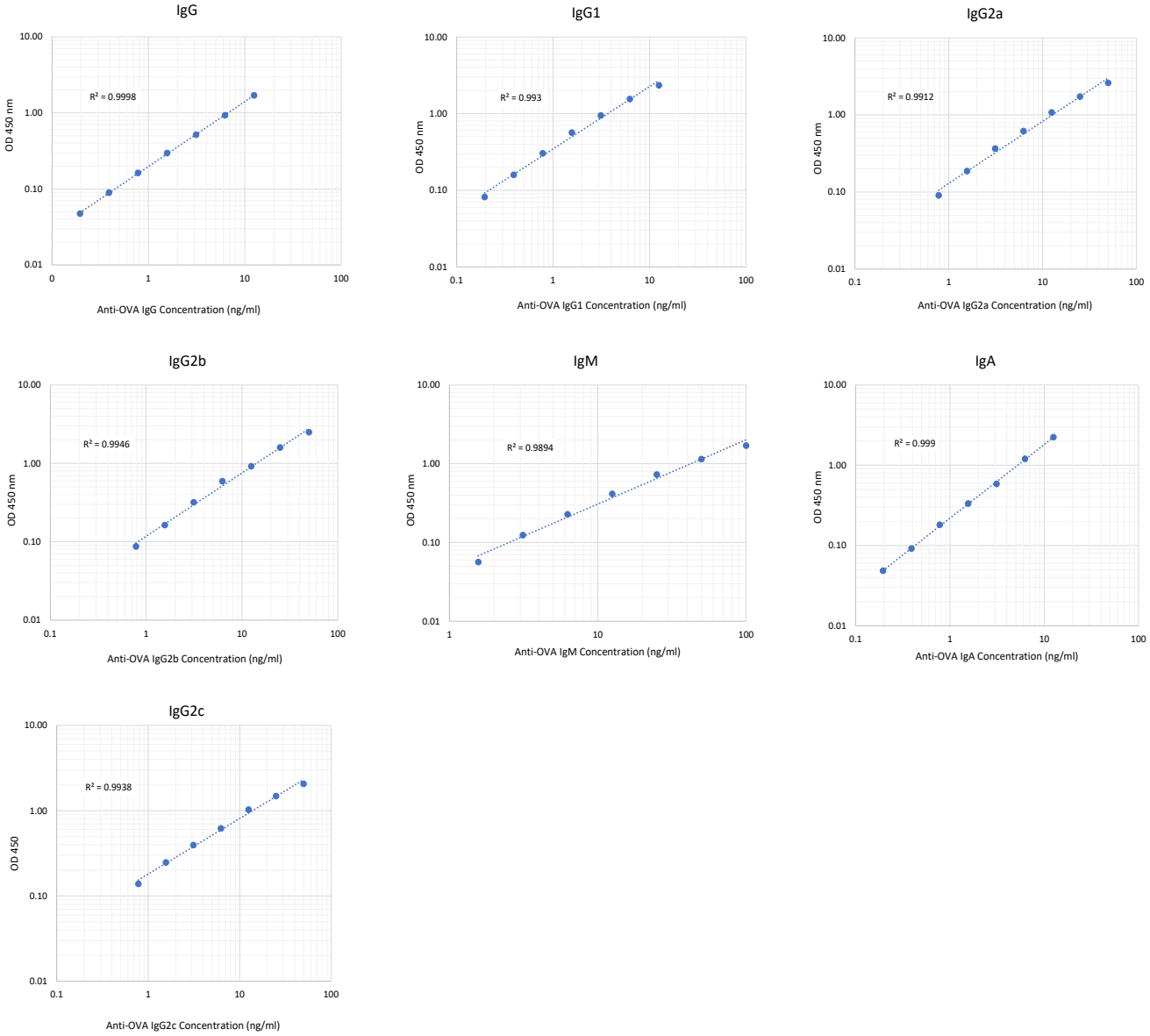
Figure 1 - Typical Standard Curves for the Anti-OVA Antibody ELISA Kits



VALIDATION DATA

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

Test	**2.1 ng/ml**	**4 ng/ml**	**8.8 ng/ml**
Intra-Assay CV (%)	9.5	5.2	2.6
Inter-Assay CV (%)	3.7	0.4	3.3
Test	**0.4 ng/ml**	**1.6 ng/ml**	**6.3 ng/ml**
Spike Test* (%)	107%	95%	93%

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Table 2 - Reproducibility Data for the Mouse Anti-OVA IgG1 Antibody ELISA Kit

Test	0.4 ng/ml	1.6 ng/ml	6.3 ng/ml
Intra-Assay CV (%)	9.1	6.3	4.7
Inter-Assay CV (%)	3.6	9.4	9.5
Spike Test* (%)	114%	105%	96%

Table 3 - Reproducibility Data for the Mouse Anti-OVA IgG2a Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	5.9	8.3	8.9
Inter-Assay CV (%)	9.4	8.6	6.3
Spike Test* (%)	102%	104%	98%

Table 4 - Reproducibility Data for the Mouse Anti-OVA IgG2b Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	9.9	9.5	3.9
Inter-Assay CV (%)	9.4	8.6	6.3
Spike Test* (%)	104%	106%	94%

Table 5 - Reproducibility Data for the Mouse Anti-OVA IgM Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	2.9	4.6	4.4
Inter-Assay CV (%)	6.6	9.1	9.0
Spike Test* (%)	112%	116%	93%

Table 6 - Reproducibility Data for the Mouse Anti-OVA IgA Antibody ELISA Kit

Test	2 ng/ml	4 ng/ml	8 ng/ml
Intra-Assay CV (%)	7.8	0.2	2.1
Inter-Assay CV (%)	7.9	1.2	5.2
Spike Test* (%)	97%	98%	93%

Table 7 - Reproducibility Data for the Mouse Anti-OVA IgG2c Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	5.8	9.6	5.5
Inter-Assay CV (%)	7.0	4.0	2.9
Spike Test* (%)	101%	109%	100%

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

TROUBLESHOOTING

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

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