Ovalbumin Detection ELISA Kit

Catalog # 6032

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify ovalbumin
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	100 - 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Culture media, serum, plasma, and solubilized samples (extracts)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (9.1-10%) /Inter-Assay (1.5-10.8%) /Spiking Test (90-96%)
NOTES:	

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INTRODUCTION

Allergic diseases and symptoms arise from an active immune response to antigens which are usually harmless, such as pollen, pet dander, or food. Food allergies and asthma are two common childhood autoimmune diseases that have recently begun to affect more individuals. A food allergy is an immune response to foods or food ingredients that most other people can tolerate with no problem. Chicken egg allergy is the second most common food allergy (the first being bovine milk allergy) and is observed in 0.5 to 2.5% of young children. Egg allergy is an immunological reaction induced by egg proteins and is defined as an allergen-specific IgE antibody-mediated allergy, also known as a type I food allergy. Egg white contains several allergens such as ovalbumin (OVA), ovomucoid, ovotransferin and lysozyme. OVA makes up about 60% of the total protein in egg white(1, 2)).

OVA generally is consumed after thermal processing. Studies have shown that heating alters both its molecular structure and allergenicity. (3) . However, it should be considered that egg allergens are processed at different temperatures (baked, scrambled or soft/hard boiled eggs or even native as whipped egg white) and these processing conditions can have a major impact on the secondary structure, susceptibility to enzymatic digestion in the gastrointestinal tract and allergenicity. Moreover, heating of allergens can lead to their aggregation, which reduces their absorption and transport through epithelial layer and thus decreases their allergenicity.

Several methods have been developed for the identification and quantification of food allergens, including high performance liquid chromatography, polymerase chain reaction, mass spectrometry (MS), surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA). ELISA can provide high sensitivity and specificity without sophisticated equipment, and it is the most commonly used method for food allergen analysis. Various ELISA-based methods have been developed for the detection of several food allergens (4).

To analyze the pathogenesis of OVA, in allergic reactions in patients or animal disease models as well as quality test of allergen contamination in foods, Chondrex, Inc. provides allergen detection ELISA kits. For more information, please visit www.chondrex.com or contact support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
OVA Standard (60321)	1 vial	100 ng, lyophilized	-20°C
Detection Antibody (60323)	1 vial	100 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	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 (90023)	2 vials	200 µl	-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
Capture Antibody Coated 96-Well ELISA Plate (Orange)	1 each	8-well Strips x12	-20°C

ASSAY OUTLINE

Add 100 µl of diluted standards and samples into wells

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

Add 100 µl of diluted Detection Antibody

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

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of TMB solution

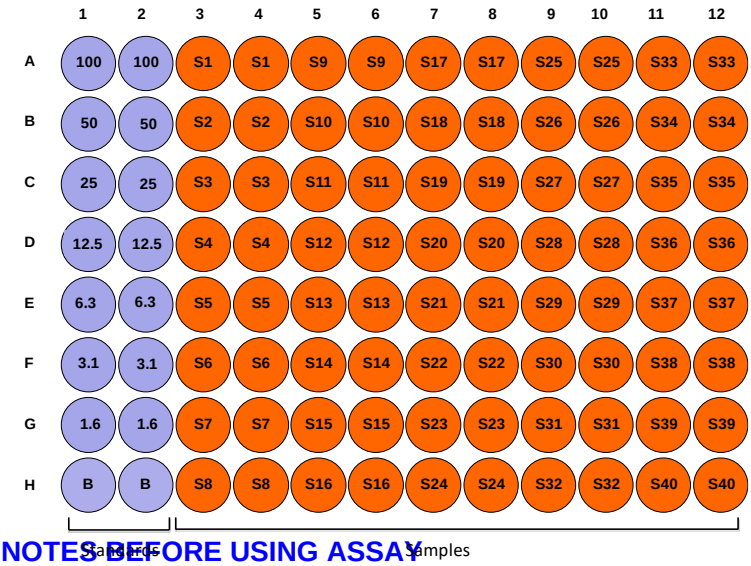
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



NOTES BEFORE USING ASSAY

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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.

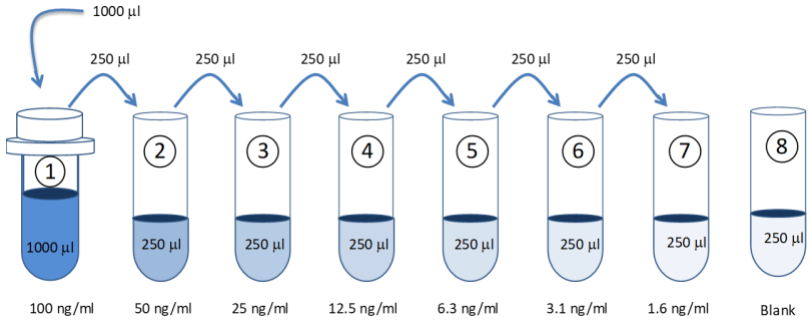
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 1.6 - 100 ng/ml. Dissolve one vial of OVA standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 100 ng/ml standard. 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 solutions. The remaining 100 ng/ml standard stock may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



2. **Prepare Samples:** Dilute samples at least 1:1 with Solution B depending on the estimated OVA level in the samples. Two to three different sample dilutions are recommended if the OVA levels in the samples are unknown.

   NOTE: Samples must be diluted with Solution B to maintain optimal assay conditions.

3. **Add Standards and Samples**: Add 100 µl of Solution B (blank), standards, and samples to designated wells in duplicate and incubate at room temperature for 2 hours.

4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X 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 Detection Antibody Solution**: Prepare the detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown 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 B (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

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 Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	41	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 TMB Solution**: Dilute one vial of TMB in 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of TMB solution to all wells 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

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **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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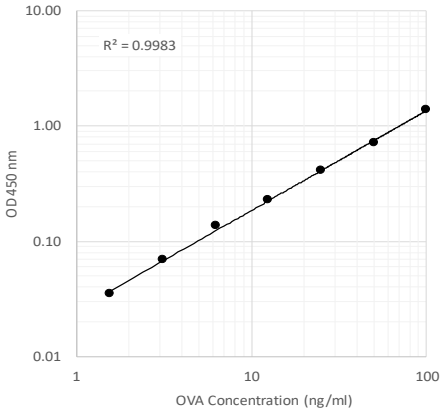
CALCULATING RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the "blank" (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of OVA (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 OVA in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the OVA Detection ELISA Kit



ASSAY VALIDATION

Table 1 – Reproducibility Data for the OVA Detection ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	9.5	10.0	9.1
Inter-Assay CV (%)	10.8	8.5	1.5
Spike Test* (%)	96%	90%	95%

* Known amounts of OVA were added to samples and then diluted with Sample/Standard/Detection Antibody 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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1. J.-C. Caubet, J. Wang, Current understanding of egg allergy. Pediatr. Clin. North Am. 58, 427–43, xi (2011).
2. M. P. Borres, N. Maruyama, S. Sato, M. Ebisawa, Recent advances in component resolved diagnosis in food allergy. Allergol. Int. 65, 378–387 (2016).
3. J. Golias, M. Schwarzer, M. Wallner, M. Kverka, H. Kozakova, D. Srutkova, K. Klimesova, P. Sotkovsky, L. Palova-Jelinkova, F. Ferreira, L. Tuckova, Heat-induced structural changes affect OVA-antigen processing and reduce allergic response in mouse model of food allergy. PLoS One 7, e37156 (2012).
4. J. Peng, X. Meng, X. Deng, J. Zhu, H. Kuang, C. Xu, Development of a monoclonal antibody-based sandwich ELISA for the detection of ovalbumin in foods. Food Agric. Immunol. 25, 1–8 (2014).

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