# Ovalbumin Detection Kit

Catalog # 6032

*For Research Use Only - Not Human or Therapeutic Use*

## INTRODUCTION

Allergic diseases and symptoms arise from an active immune response to antigens which are usually harmless, such as pollen, pet dander, or food. Specifically, food allergies and asthma are two common childhood autoimmune diseases that have affected more individuals recently.

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. Among egg proteins, ovalbumin (OVA) is one of the identified allergenic proteins (1) (2).

Asthma is a chronic inflammatory disease that affects 300 million people of all ages worldwide (3). It is caused by exposure to allergens such as dust mites, pet dander, pollen, or mold, and characterized by airflow obstruction and bronchospasm. House dust mite (HDM) allergens are the most important indoor allergens in humans (3). Approximately 10% of asthma patients sensitized to HDM demonstrate IgE antibodies to the tropomyosin protein (Der p10) from dust mite, *Dermatophagoides pteronyssius* (2). Der p10 is a 32 kDa, group 10 allergen (tropomyosins). Tropomyosins of a similar structure are found in invertebrates such as crustaceans (shrimp, lobster, crawfish, and crab), arachnids (house dust mites), insects (cockroaches), and mollusks. Tropomyosin is a heat-stable protein and sensitization may lead to severe reactions to the allergenic source. Therefore, the Der p10 antigen may play a role in the cross-induction of allergic reaction to many allergens. (4)

To analyze the pathogenesis of these allergens, OVA and HDM (Der p 10), in allergic reactions in patients or animal disease models, Chondrex, Inc. provides allergen detection ELISA kits: OVA (Cat # 6032) and HDM (Der p 10) (Cat # 6031). For more information, please contact Chondrex, Inc. at 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 | 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 |
| Capture Antibody Coated 96-Well ELISA Plate | 1 each | 8-well strips x 12 | -20°C |



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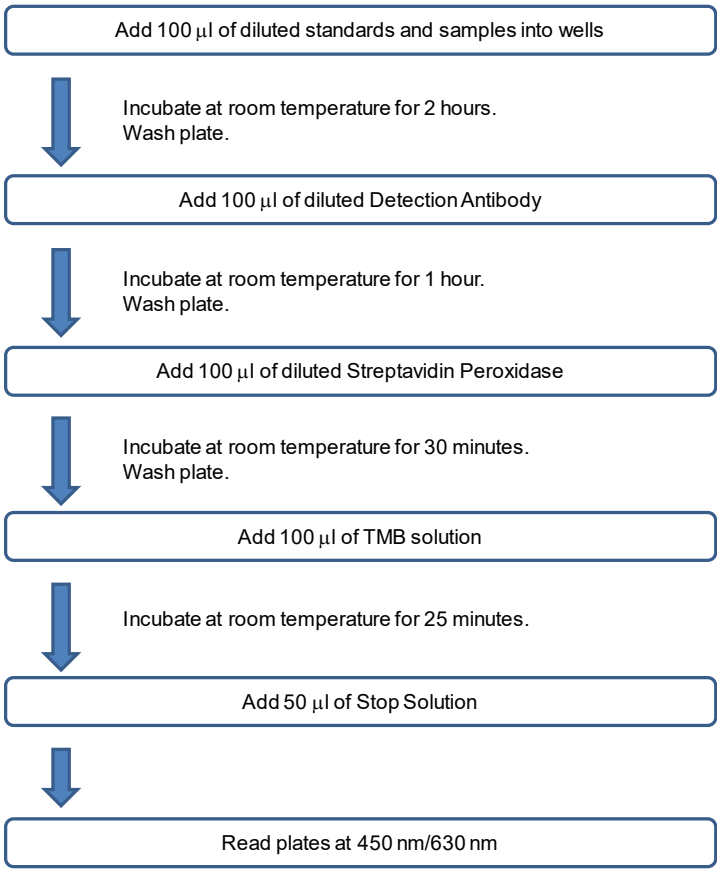
## ASSAY OUTLINE




## 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: Partially used reagents may be kept at –20°C.

Note 4: 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 dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.



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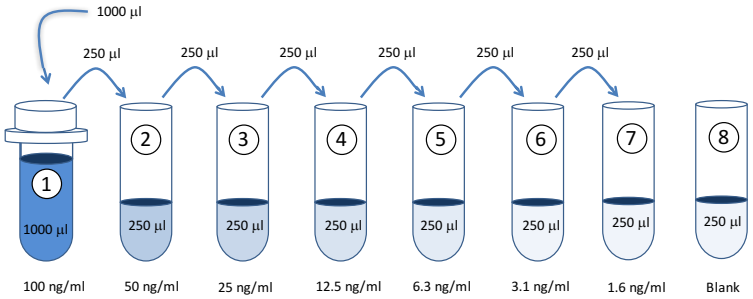
## ASSAY PROCEDURE

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. We recommend making fresh serial dilutions for each assay.




2. **Prepare Samples**:

   **Dilution**: 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 according to the layout in Figure 1. Incubate at room temperature for 2 hours.

Figure 1 - A Standard Assay Layout

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

5. **Add Detection Antibody Solution**: Prepare detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown in the following table.

| 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 |

Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

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 blot 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.

| Strip # | Streptavidin Peroxidase (µl) | Solution D (ml) |
|---|---|---|
| 2 | 8 | 1.8 |
| 4 | 17 | 3.4 |
| 6 | 25 | 5.0 |
| 8 | 33 | 6.6 |
| 10 | 41 | 8.2 |
| 12 | 50 | 10.0 |

Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **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.

| 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 |

Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

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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## CALCULATION OF 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 2 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 2 - A typical standard curve for OVA assay

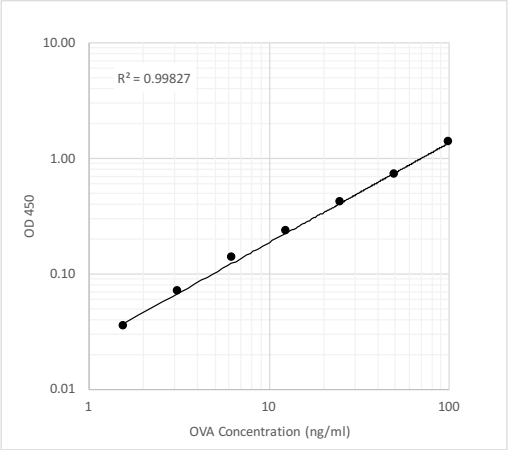


Table 1 - Reproducibility for OVA ELISA Kit

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

* Known amounts of OVA was added to a 1% BSA/PBS solution and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

## REFERENCES

1. J. Caubet, J. Wang, Current Understanding of Egg Allergy. *Pediatr Clin North Am* **58**, 427-43, xi (2011).
2. M. Lloyd, Advances in Allergy Testing: Component Resolved Diagnostics. *Journal for Clinical Studies*, 1–4 (2017).
3. T. Buday, J. Plevkova, House Dust Mite Allergy Models - Reliability for Research of Airway Defensive Mechanisms. *OJMIP* **04,** 27–35 (2014).
4. R. H. Shafique, M. Inam, M. Ismail, F. R. Chaudhary, Group 10 allergens (tropomyosins) from house-dust mites may cause covariation of sensitization to allergens from other invertebrates. *Allergy Rhinol (Providence)* **3,** e74–90 (2012).



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