Mouse Anti-OVA IgM Antibody Assay Kit

Catalog # 3017

For Research Use Only - Not Human or Therapeutic Use

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 isotype and subtype anti-OVA mouse antibody assay kits. Investigators studying the pathogenesis of allergic diseases in mice should consider several issues such as mucosal immunity, antibody responses to different isotypes (e.g. IgA, IgE, IgG, and IgM) and subtypes (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 functionmay 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 antibody isotype in serum, protects the host from pathogens and unwanted antigens that have penetrated into the body. On the other hand, IgA, a known mucosal immunoglobulin, prevents the penetration of pathogens and other unwanted antigenic substances through mucosal membrane. 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 isotypes and subtypes 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 isotypes. For example, alum 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).

Chondrex, Inc. provides the following ELISA kits to study the mouse antibody responses against OVA. Each kit uses a corresponding isotype or subtype antibody standard.

List of mouse anti-OVA antibody ELISA kits:

1. Mouse Anti-OVA IgE Antibody Assay Kit (catalog # 3004)
2. Mouse Total IgE (IgEa and IgEb) Detection Kit (catalog # 3005)
3. Mouse Serum Anti-OVA IgE Antibody Assay Kit (catalog # 3010)
4. Mouse Anti-OVA IgG Antibody Assay Kit (catalog # 3011)
5. Mouse Anti-OVA IgG1 Antibody Assay Kit (catalog # 3013)
6. Mouse Anti-OVA IgG2a Antibody Assay Kit (catalog # 3015)
7. Mouse Anti-OVA IgG2b Antibody Assay Kit (catalog # 3016)
8. Mouse Anti-OVA IgG2c Antibody Assay Kit (catalog # 3029)
9. Mouse Anti-OVA IgM Antibody Assay Kit (catalog # 3017)
10. Mouse Anti-OVA IgA Antibody Assay Kit (catalog # 3018)

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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgM Antibody	1 vial	1000 ng/vial, lyophilized	-20°C
Ovalbumin (OVA)	1 vial	100 μg/vial, lyophilized	-20°C
Detection Antibody (Peroxidase-Conjugated Goat Anti-Mouse IgM Immunoglobulin Polyclonal Antibody)	2 vials	50 μl	-20°C
Solution A - OVA Dilution Buffer	1 bottle	10 ml	-20°C
Solution B - Blocking Buffer	1 bottle	10 ml	-20°C
Solution C - Sample/Standard/Detection Antibody Dilution Buffer	1 bottle	50 ml	-20°C
TMB Solution	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 ml	-20°C
Wash Buffer, 20X	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: Partially used reagents may be kept at –20°C.

Note 4: Crystals may form in the 20X wash buffer 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 pipette, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to avoid the edge effect.

ASSAY PROCEDURE

1. **Add OVA Solution**: Dissolve one vial of Ovalbumin (OVA) with 10 ml of OVA Dilution Buffer (Solution A). Add 100 μl of OVA solution to each well and incubate at 4°C overnight.

2. **Dilute Wash Buffer**: 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 blot 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.

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4. **Prepare Standard Dilutions**: The recommended standard range is 1.6-100 ng/ml. Dissolve one vial of Standard (1000 ng/vial) in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution C) and keep it as a standard stock. Add 50 µl of this standard solution to 450 µl of Solution C to make a 100 ng/ml solution. Then, serially dilute it with Solution C. For example, mix 250 µl of the 100 ng/ml solution with an equal volume of Solution C 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 standard solutions.

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 IgM antibodies against OVA are observed in normal serum at a 1:10 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 blot 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.

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

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

11. **Add TMB**: Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml 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.

12. **Stop**: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

13. **Read Plate**: Read the OD values at 450 nm (a 630 nm filter can be used as a reference). If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

CALCULATION OF ANTIBODY TITERS

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

2. Subtract the averaged blank (B) 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 of anti-OVA IgM subtype 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 sample specimens.

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Figure 1 - A typical standard curve for mouse anti-OVA IgM assay

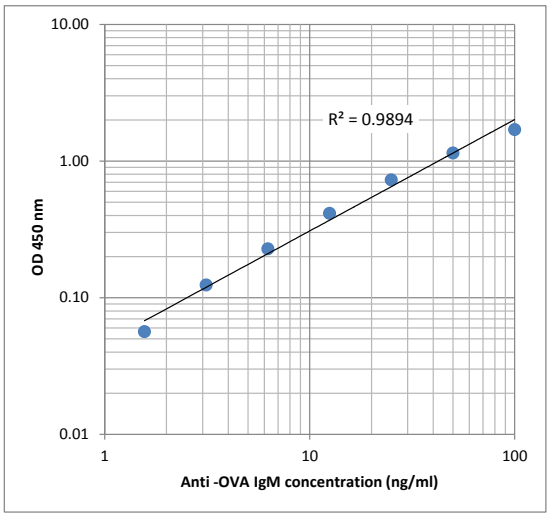

Table 1 - Reproducibility of data assayed by Mouse Anti-OVA IgM Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	6.6	9.1	9.0
Intra-Assay CV (%)	2.9	4.6	4.4
Spiking Test*	111.2%	115.1%	93.0%

Standard was added with known amounts of IgM and then diluted with Sample/Standard/Detection Antibody Dilution Buffer to assay anti-OVA IgM antibodies by ELISA.

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