

## Mouse Total IgA Antibody Detection Kit

Catalog # 3019

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

### INTRODUCTION

The total IgA levels in specimens are often determined in mouse disease models involving allergy (1), virus infection (2), nephritis (3), inflammatory bowel disease (4), and agammaglobulinemia (5,6). Moreover, total immunoglobulin isotype values (IgG, IgM, IgA, or IgE) in the sample can be compared to anti-antigen specific isotype antibody levels, such as anti-ovalbumin (OVA) antibodies. Ovalbumin (OVA) is a widely used antigen for inducing allergic reactions in experimental animals (7-10). As such, Chondrex provides Mouse Total IgA Antibody Detection Kit for the quantification of IgA in samples such as serum and cultured mouse cells.

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 system 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, 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 (11).
- 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 are 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 and IgG2b are dependent on Th1 cells. Thus, to investigate the immune responses involved in allergic reactions in OVA-induced allergic mouse models (7-10), anti-OVA IgE, IgG, and IgA antibody ELISA kits are valuable tools.
- 3) **Adjuvants:** The type of adjuvant can elicit specific antibody isotypes. For example, alum adjuvant is widely used to elicit IgE antibodies (12), whereas Cholera toxins are effective at eliciting IgA antibodies (13). Moreover, Complete Freund’s Adjuvant (CFA) is widely used for stimulating IgG and IgM antibody production (14).

Chondrex provides the following ELISA kits to study the mouse antibody responses against OVA, as well as total IgE and IgA ELISA kits. 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 IgM Antibody Assay Kit (catalog # 3017)
9. Mouse Anti-OVA IgA Antibody Assay Kit (catalog # 3018)
10. Mouse Total IgA Detection Kit (catalog # 3019)

## KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse IgA Antibody	1 vial	1000 ng/vial, lyophilized	-20°C
Capture Antibody (Goat Anti-Mouse IgA Immunoglobulin Polyclonal Antibody)	1 vial	100 µl	-20°C
Detection Antibody (Peroxidase-Conjugated Goat Anti-Mouse IgA Immunoglobulin Polyclonal Antibody)	1 vial	100 µl	-20°C
Solution A - Capture Antibody Dilution Buffer	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer	1 bottle	50 ml	-20°C
Solution C - Detection Antibody Dilution Buffer	1 bottle	10 ml	-20°C
OPD	2 vials	Lyophilized	-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 prevent evaporation from the outside wells of the plate.

## ASSAY PROCEDURE

- Add Capture Antibody:** Dilute one vial of Capture Antibody with 10 ml of Capture Antibody Dilution Buffer (Solution A). Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight.
- 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.*
- 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 Dilution Buffer (Solution B) and keep it as a standard stock. Add 50 µl of the standard (1000 ng/ml) with 450 µl of Solution B to make a 100 ng/ml solution. Then serially dilute it with Solution B. For example, mix 250 µl of the 100 ng/ml solution with an equal volume of Solution B to make a 50 ng/ml solution, and then repeat it five more times to make 25, 12.5, 6.3, 3.1, and 1.6 ng/ml solutions.

4. **Prepare Sample Dilutions:** The adjuvant used for immunization and the timing of serum collection may influence the level of IgA in serum. Several dilutions of your sample to ensure that the sample OD values are within the standard curve range are recommended. In general, a 1:2000 dilution can be used for normal mice.
5. **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.*
6. **Add Standards and Samples:** Add 100  $\mu$ l of standards, Solution B (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.
7. **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.*
8. **Add Detection Antibody:** Dissolve one vial of Detection Antibody in 10 ml of Detection Antibody Dilution Buffer (Solution C). Add 100  $\mu$ l of detection antibody solution to each well and incubate at room temperature for 1 hour.
9. **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.*
10. **Add OPD:** Dissolve one vial of OPD with 10 ml Chromagen Dilution Buffer just prior to use. Add 100  $\mu$ l of OPD solution to all wells immediately after washing the plate and incubate for 25 minutes at room temperature.
11. **Stop:** Add 50  $\mu$ l of 2N sulfuric acid (Stop Solution) to each well.
12. **Read Plate:** Read the OD values at 490 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 IgA antibodies.
4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply by the sample dilution factor to obtain the antibody concentration (ng/ml) in original sample specimens.

Figure 1 - A typical standard curve for mouse total IgA assay

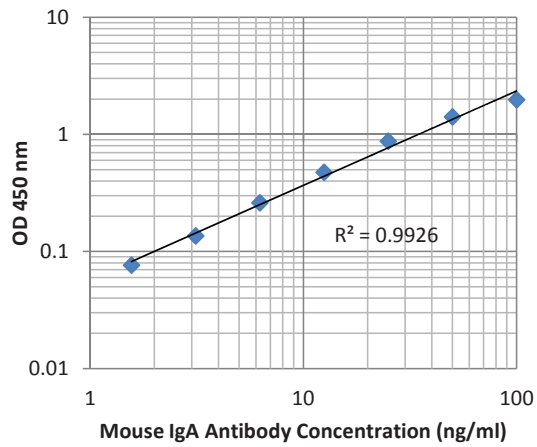


Table 1 - Reproducibility of data assayed by Mouse Total IgA Antibody Detection Kit

Test At	10 ng/ml	20 ng/ml	40 ng/ml
Inter-Assay CV (%)	6.7	8.5	3.3
Intra-Assay CV (%)	7.9	3.2	6.3
Spiking Test*	102%	104%	103%

Standard was added with known amounts of IgA and then diluted with Sample/Standard Dilution Buffer to assay total IgA antibodies by ELISA.

## REFERENCES

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