Chondrex, Inc.

Mouse Serum Anti-HDM IgE Antibody Assay Kit

Catalog # 3037

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INTRODUCTION

Asthma is a common chronic inflammatory disease that affects 300 million people of all ages worldwide (1). 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) is the most common asthma allergen, which affects up to 85% of asthma patients (2, 3). Of the two main mite species, *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f), more than 20 types of HDM allergens are defined based on sequential and functional homologies. Among those HDM allergens, group 1 (Der 1) and group 2 (Der 2) dominate overall allergic responses in patients and are the most commonly researched allergens (4-6).

Previously, asthma was considered to be an inflammatory airway disease mediated by the adaptive immune system, particularly type 2 helper T-cells (7). However, recent studies indicate that the innate immune system is also involved in triggering an inflammatory response in both asthma patients and animal models (8-10). These inflammatory changes and airway remodeling significantly vary depending on the types of allergens (11). To meet such needs, a mouse HDM-induced asthma model is a useful tool to dissect the pathological roles of the adaptive and innate immune systems activated by different HDM elements. This is an advantage over the classic ovalbumin-induced asthma model which activates adaptive immunity preferentially.

Recently, it was reported that HDM-specific sublingual immunotherapy (SLIT) is more efficacious than subcutaneous immunotherapy at preventing the development of allergic inflammatory reactions in a mouse model (12). This SLIT protocol has been approved as a treatment to reduce allergy or asthma symptoms in patients (13).

In general, mouse serum antigen-specific antibody levels for antibodies like IgA and IgG tend to be higher than IgE levels. Thus it is difficult to detect antigen-specific IgE antibody levels due to the competition for the antigenic determinant on the antigen by other types of antibodies. This kit (catalog # 3037) is designed to detect HDM specific IgE antibodies in mouse sera and works for both IgE^a (Balb/c) and IgE^b (C57BL/6) allotypes equally.

To study the immune response to allergens and allergen-specific pathological effects in mouse models, Chondrex, Inc. provides mouse ELISA kits for assying anti-HDM IgG, IgM , and IgE antibodies (Catalog # 3030, 3036, 3037), in addition to anti-HDM antibody IgG subclasses IgG1, IgG2a, IgG2b, and IgG3 (Catalog # 3034, 3038, 3035, 3039). Chondrex, Inc. also offers ELISA kits for assaying anti-OVA antibody subtypes IgA, IgE, IgG, and IgM and anti-OVA IgG subclasses IgG1, IgG2a, IgG2b, and IgG2c, (Catalog # 3004, 3010, 3011, 3015 - 3018, 3029) as well as total immunoglobulin subtypes IgA, IgE, IgG, and IgM, and IgG subclasses IgG1, IgG2a, IgG2b, and IgG3, (Catalog # 3005, 3019, 3023 - 3028). Other antibody subtype ELISA kits against HDM as well as HDM allergen detection kits are currently under development. Please contact Chondrex, Inc. support (support@chondrex.com) for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Anti-HDM Mouse IgE Antibody (30371)	1 vial	50 ng/vial, lyophilized	-20°C
Biotinylated HDM (30373)	1 vial	100 µl	-20°C
Solution C - Sample/Standard Dilution Buffer (30314)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Solution E- Biotinylated HDM Dilution Buffer (30374)	1 bottle	10 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-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
Anti-Mouse IgE Antibody Coated ELISA Plate	1 each	96-well (Yellow 8-well strips x 12)	-20°C

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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 completely dissolved.

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

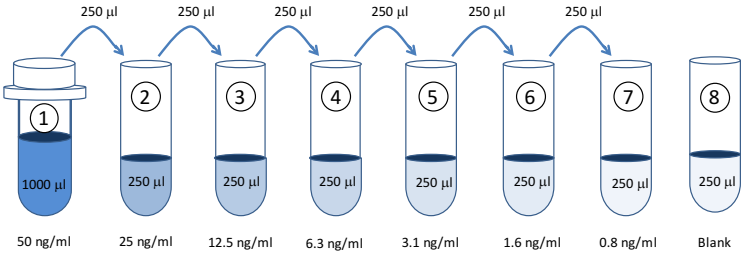
Note 6: Cover the plate with plastic wrap or a plate sealer after each step to avoid the edge effect.

Note 7: Serum IgE antibodies are a mixture of multiple antibodies with a variety of affinity ranges. The OD value obtained in ELISA for an antibody assay depends on the antibody concentration as well as the antibody affinity towards an antigen. In general, ELISA data using a monoclonal antibody as a standard does not reflect the absolute levels of IgE. Therefore, IgE levels determined using this kit should be expressed as ng of IgE per ml.

Note 8: If the total IgE concentration in a sample is higher than 500 ng/ml, the sample must be diluted to lower the total IgE levels below 500 ng/ml because the anti-HDM IgE value obtained from this ELISA is lower than the actual value due to competition from non-anti-HDM IgE antibodies in sample (Figure 3). Therefore, it is strongly recommended that total IgE levels be determined first using the Mouse Total IgE Assay Kit (Catalog # 3005).

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.8 - 50 ng/ml. Dissolve one vial of Standard (50 ng/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution C) and keep it as a 50 ng/ml standard stock. Add 250 µl of this standard solution to 250 µl of Solution C to make a 25 ng/ml solution. Then, serially dilute it with Solution C. For example, mix 250 µl of the 50 ng/ml solution with an equal volume of Solution C to make a 25 ng/ml solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, and 0.8 ng/ml standard solutions.



2. **Prepare Sample Dilutions**: The dilution of serum from mouse immunized with HDM varies (1:10 or more) depending on the immunization schedule and timing of serum collection. In general, no IgE antibodies against HDM are observed in normal serum at a 1:10 dilution.

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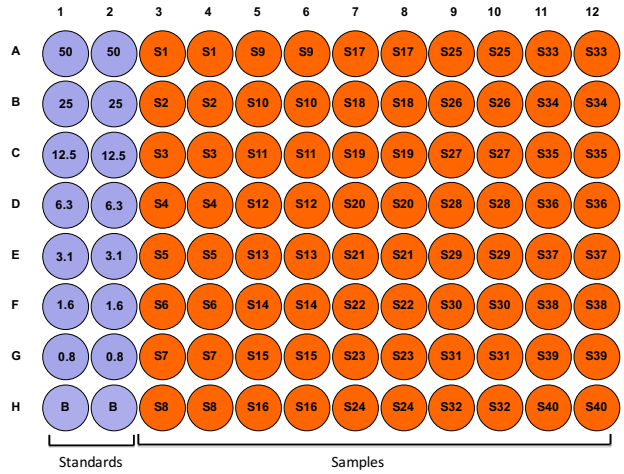
3. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

Figure 1 - Mouse serum anti-HDM IgE Assay Standard layout.



4. **Dilute Wash Buffer:** Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer).

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 Biotinylated HDM**: Dilute one vial of biotinylated HDM in 10 ml Biotinylated HDM Dilution Buffer (Solution E). Add 100 µl of detection antibody solution to each well and 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 Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

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 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.

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

12. **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 2 shows an example of a standard curve of anti-HDM IgE 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.

Figure 2 - A typical standard curve for anti-HDM IgE assay

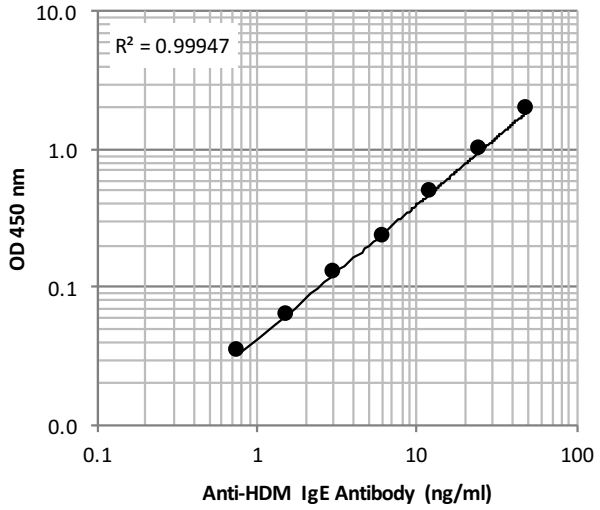
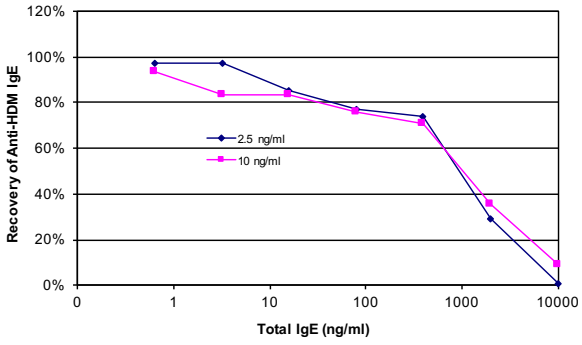


Table 1 - Reproducibility of data assayed by Mouse Anti-HDM IgE Antibody Assay Kit

Test At	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Inter-Assay CV (%)	5.3	5.1	3.2
Intra-Assay CV (%)	5.0	2.7	1.7
Spiking Test*	90%	90%	103%

Known amounts of anti-HDM IgE were added to samples and then diluted with Sample/Standard Dilution Buffer to assay anti-HDM IgE antibodies by ELISA.

Figure 3 - Influence of non-anti-HDM IgE antibodies in samples



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