Mouse Anti-HDM *Dermatophagoides pteronyssinus* & *Dermatophagoides farinae* Serum IgE Antibody Detection ELISA Kits

Catalog # 3037 and 3081

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-house dust mite (HDM) *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* house dust mite IgE antibodies
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5 hours
STANDARD RANGE:	50 - 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:10 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	HDM DP: Intra-Assay (1.7-5%)/Inter-Assay (3.2-5.3%)/Spiking Test (90-103%)
	HDM DF: Intra-Assay (2.5-3.4%)/Inter-Assay (2.5-3.0%)/Spiking Test (96-106%)
NOTES:	N/A

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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: DP) and *Dermatophagoides farinae* (Der f: DF), 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 researched allergens (4-6).

Previously, asthma was considered 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). Airway remodeling and inflammatory changes 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 the different HDM elements. This is an advantage over the classical ovalbumin-induced asthma model which preferentially activates adaptive immunity.

Recently, it was reported that HDM-specific sublingual immunotherapy (SLIT) is more efficacious at preventing the development of allergic inflammatory reactions than subcutaneous immunotherapy in mouse models (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 is designed to detect HDM specific IgE antibodies in mouse serum 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 assaying IgE antibodies against HDM DP (Catalog # 3037) and HDM DF (Catalog # 3081) as well as other subtypes/subclasses. Chondrex, Inc. also offers ELISA kits for assaying antibody subtypes/subclasses against many kinds of allergens as well as total immunoglobulin subtypes/subclasses. Please visit www.chondrex.com for more information.

Note: Other antibody subtype ELISA kits against HDM as well as HDM antigen detection kits are currently under development. Please contact Chondrex, Inc. at support@chondrex.com for more information.

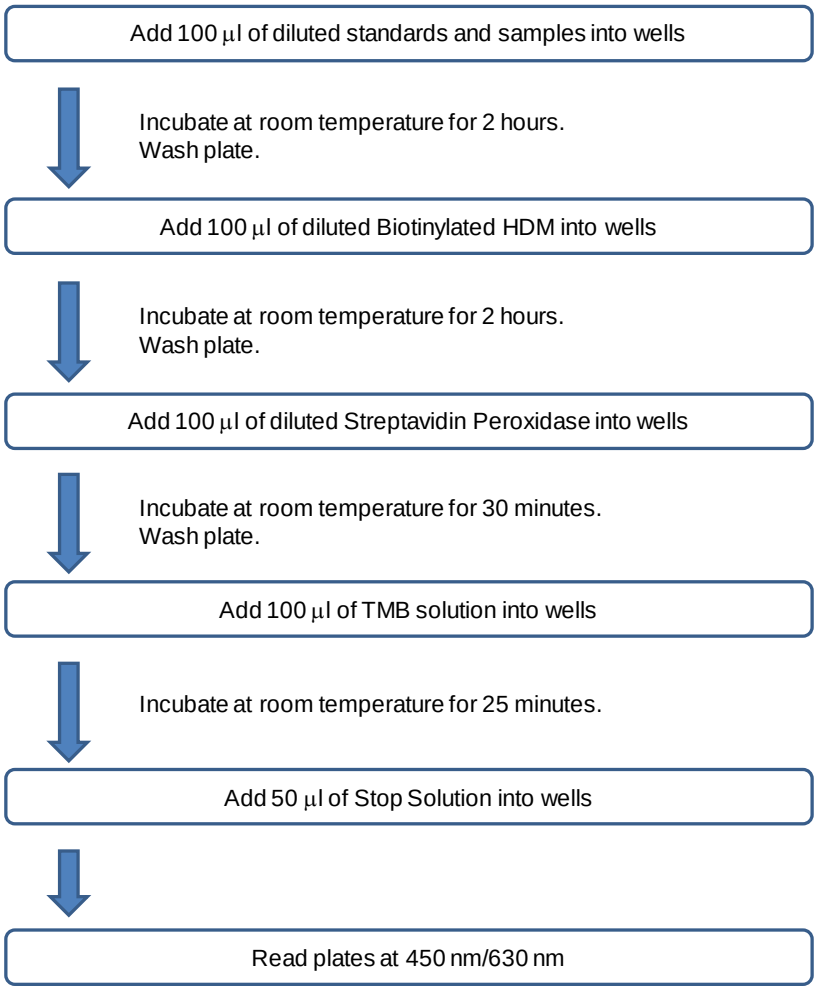
LIST OF MOUSE ANTI-HDM ANTIBODY SUBTYPE/SUBCLASS ELISA KITS

Kit	Der p Catalog #	Der f Catalog #
Mouse Anti-HDM IgG Antibody ELISA Kit	3030	3072
Mouse Anti-HDM IgG1 Antibody ELISA Kit	3034	3073
Mouse Anti-HDM IgG2b Antibody ELISA Kit	3035	3074
Mouse Anti-HDM IgM Antibody ELISA Kit	3036	3076
Mouse Anti-HDM IgE Antibody ELISA Kit	3037	3081
Mouse Anti-HDM IgG2a Antibody ELISA Kit	3038	Coming soon!
Mouse Anti-HDM IgG3 Antibody ELISA Kit	3039	3075
Mouse Anti-HDM IgA Antibody ELISA Kit	3046	Coming soon!

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Anti-HDM Mouse IgE Antibody (30371)	1 vial	50 ng, lyophilized	-20°C
3037: Biotinylated HDM DP (30373) 3081: Biotinylated HDM DF (30813)	1 vial	100 µl	-20°C
Solution C - Sample/Standard/Secondary Antibody 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 (Yellow)	1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE



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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: 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 -20ºC 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.

NOTE 8: 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 9: 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 present in the sample (Figure 2). Therefore, it is strongly recommended that total IgE levels be determined first using the Mouse Total IgE Assay Kit (Catalog # 3005).

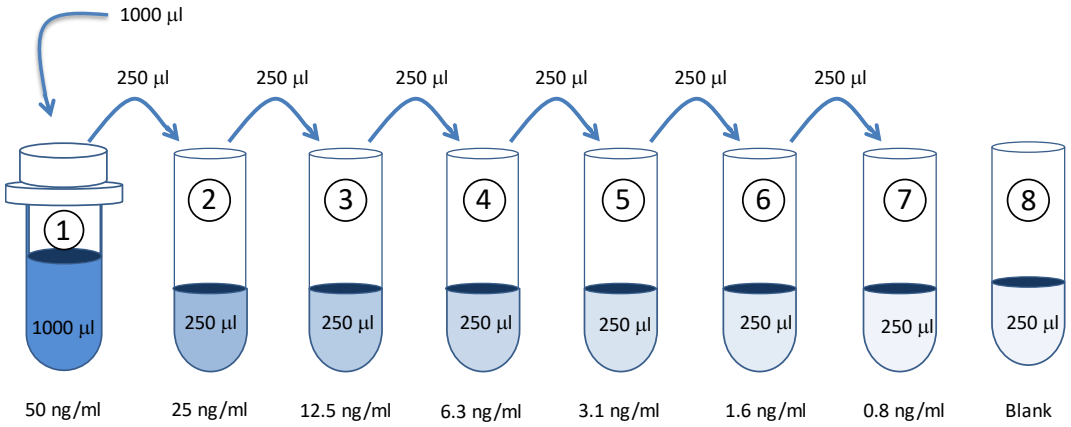
PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	50	50	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	25	25	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	12.5	12.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	6.3	6.3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	3.1	3.1	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	1.6	1.6	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.8	0.8	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

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/Secondary Antibody Dilution Buffer (Solution C) and keep it as a standard stock. 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**: An important point to note is that the composition of HDM can exhibit variations depending on the vendor, batch, and manufacturing process. These variations can result in differing levels of antigens in the final HDM product, which when used to immunize mice, can impact the serum antibody levels against those antigens. The dilution of mouse serum (1:10 or more) immunized with various HDM products and types (DP or DF) will vary 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.

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.

4. **Wash**: 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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Biotinylated HDM**: Dilute one vial of biotinylated HDM in 10 ml Biotinylated HDM Dilution Buffer (Solution E). Add 100 µl of biotinylated HDM solution to each well and incubate at room temperature for 2 hours.

Strip #	Biotinylated HDM (µl)	Solution E (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	42	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**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well 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.

CALCULATING RESULTS

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

2. Subtract the averaged blank OD 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 examples of standard curves for anti-HDM Serum 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 the original test samples.

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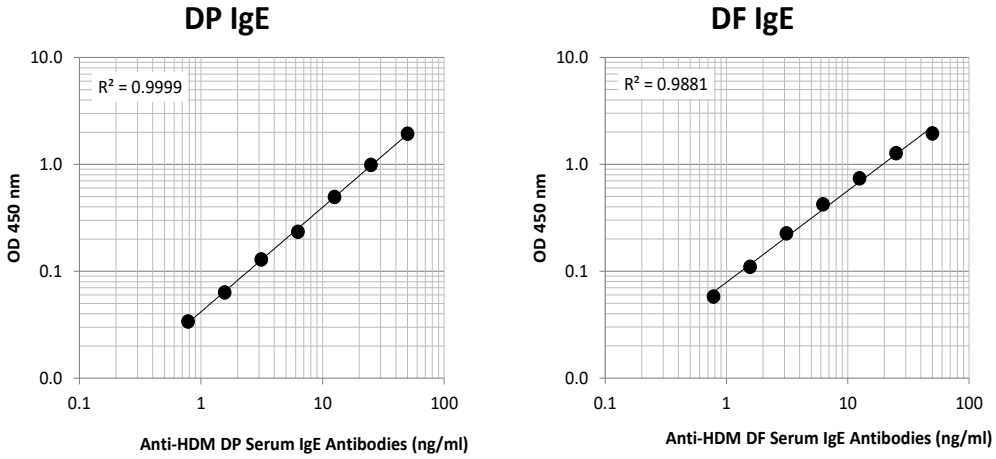
Figure 1 - Typical Standard Curves for the Anti-HDM Serum IgE Antibody ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-HDM DP Serum IgE Antibody ELISA Kit

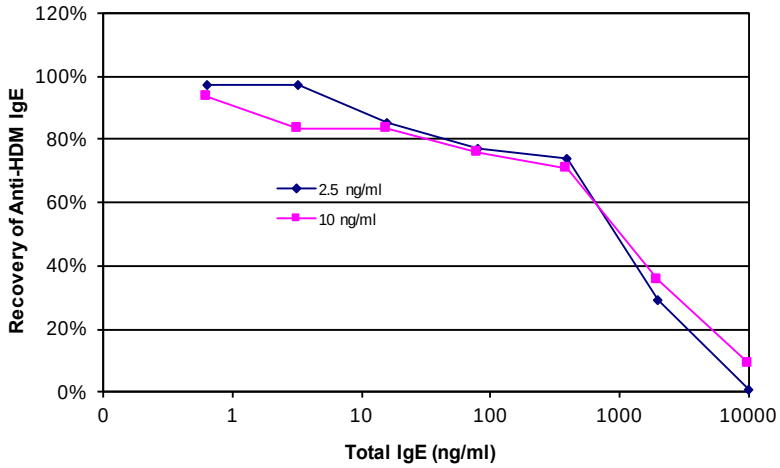
Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	5.0	2.7	1.7
Inter-Assay CV (%)	5.3	5.1	3.2
Spike Test* (%)	90%	90%	103%

Table 2 - Reproducibility Data for the Mouse Anti-HDM DF Serum IgE Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	3.4	2.5	3.0
Inter-Assay CV (%)	2.5	2.7	3.0
Spike Test* (%)	96%	100%	106%

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

Figure 2 - Influence of Non-Anti-HDM IgE Antibodies in Samples



TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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