Mouse Anti-Der p2 Antibody Subtype/Subclass ELISA Kits

Catalog # 3065, 3066, 3067, and 3068

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify mouse anti-Der p2 antibodies
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	3065 (IgG) : 12.5 - 0.2 ng/ml
	3066 (IgG2a) : 12.5 - 0.2 ng/ml
	3067 (IgG2b) : 12.5 - 0.2 ng/ml
	3068 (IgM) : 100 - 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3065: Intra-Assay (1.3-4.9%)/Inter-Assay (2.8-7.7%)/Spiking Test (94-104%)
	3066: Intra-Assay (1.2-4.1%)/Inter-Assay (2.4-10.1%)/Spiking Test (96-100%)
	3067: Intra-Assay (4.7-5.1%)/Inter-Assay (5.5-5.8%)/Spiking Test (92-95%)
	3068: Intra-Assay (0.9-2.9%)/Inter-Assay (3.3-6.3%)/Spiking Test (106-110%)
NOTES:	If serum samples require a lower dilution than 1:100, please contact support@chondrex.com

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INTRODUCTION

Asthma is a common chronic inflammatory disease that affects 300 million people of all ages worldwide (1, 2). 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 (3, 4). 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 (5–7).

Der p2 is a myeloid differentiation factor 2-related lipid-recognition protein consisting of 146 amino acids. Der p2 can activate toll-like receptor 4 on respiratory epithelial cells and thus play a role in developing respiratory diseases (8,9). In mice, Der p2 has been utilized as a potential candidate for allergy vaccine development (10), especially for DNA vaccines (11).

To study the immune response to allergens and allergen-specific pathological effects in mouse asthma models, Chondrex, Inc. provides the mouse anti-Der p2 antibody ELISA kits listed below. Chondrex, Inc. also offers ELISA kits for assaying anti-Der p1, anti-HDM, anti-Gliadin, anti-Crude Peanut Extract, and anti-Ovalbumin antibody subtypes/subclasses as well as total immunoglobulin subtypes/subclasses. Please visit www.chondrex.com for more information.

Note: Chondrex, Inc. offers both anti-HDM Der p and anti-HDM Der f ELISA kits. It is important to distinguish between these two antigens as these are two distinct species of house dust mite; there will be some degree of homology between them that can result in cross-reactivity of elicited antibodies. If HDM Der p is used to induce disease in animal models, then HDM Der p ELISA kits must be used to evaluate those animals. And if HDM Der f is used to induce disease in animal models, then HDM Der f ELISA kits must be used to evaluate those animals Other antibody subtype ELISA kits against HDM as well as HDM antigen detection kits are currently under development. Please contact Chondrex, Inc. (support@chondrex.com) for more information.

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

Kit	Der p Catalog #	Der p1 Catalog #	Der p2 Catalog #	Der f Catalog #
Mouse Anti-HDM IgG Antibody ELISA Kit	3030	3047	3065	3072
Mouse Anti-HDM IgG1 Antibody ELISA Kit	3034	3048	Coming soon!	3073
Mouse Anti-HDM IgG2b Antibody ELISA Kit	3035	Coming soon!	3067	3074
Mouse Anti-HDM IgM Antibody ELISA Kit	3036	3049	3068	3076
Mouse Anti-HDM IgE Antibody ELISA Kit	3037	Coming soon!	Coming soon!	3081
Mouse Anti-HDM IgG2a Antibody ELISA Kit	3038	Coming soon!	3066	Coming soon!
Mouse Anti-HDM IgG3 Antibody ELISA Kit	3039	3064	Coming soon!	3075
Mouse Anti-HDM IgA Antibody ELISA Kit	3046	Coming soon!	Coming soon!	Coming soon!

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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard: IgG (30651) – 12.5 ng; IgG2a (30661) – 12.5 ng; IgG2b (30671) – 12.5 ng; IgM (30681) – 100 ng	1 vial	Lyophilized	-20°C
Secondary Antibody: IgG (30113); IgG2a (30153); IgG2b (30353); IgM (30173)	2 vials	50 µl	-20°C
Solution B - Sample/Standard Dilution Buffer (30055)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (2073)	1 bottle	20 ml	-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
Der p2 from *Dermatophagoides pteronyssinus* coated ELISA Plate (Orange)	1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE

Add 100 µl of blocking buffer into wells

↓ Incubate at room temperature for 1 hour. Wash plate.

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours. Wash plate.

Add 100 µl of diluted secondary antibody solution into wells

↓ Incubate at room temperature for 1 hour. Wash plate.

Add 100 µl of TMB solution into wells

↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution into wells

↓

Read plates at 450 nm/630 nm

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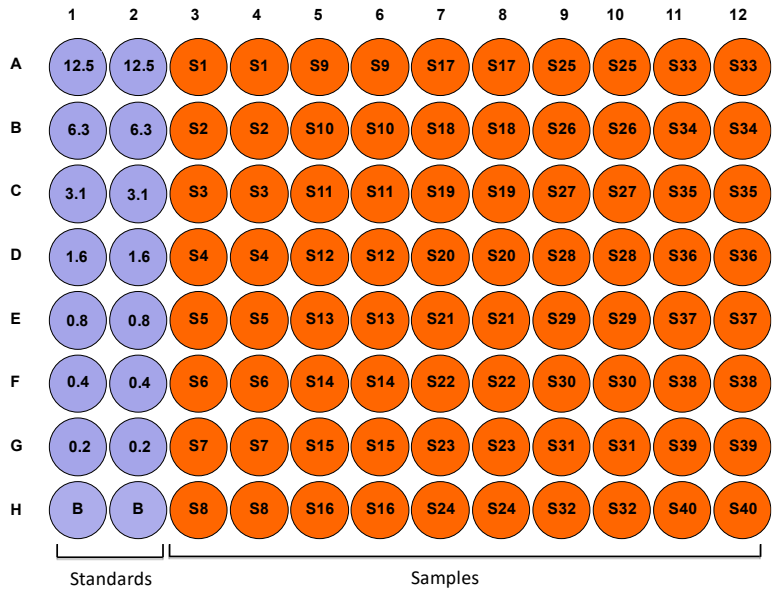
PLATE MAPPING

Example of the Mouse Anti-Der p2 IgG Antibody ELISA Kit



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 -20°C 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.

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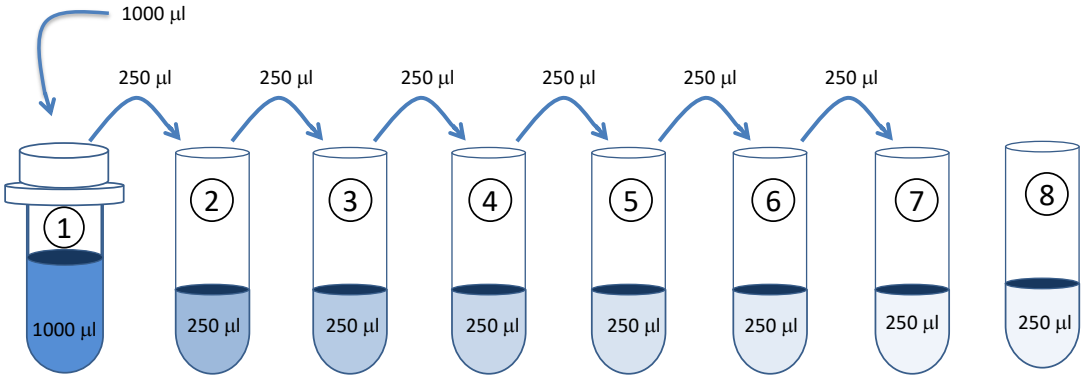
ASSAY PROCEDURE

1. **Add Blocking Buffer**: Add 100 µl of the Sample/Standard Dilution Buffer (Solution B) to each well and incubate at room temperature for 1 hour.

2. **Prepare Standard Dilutions**: Please see the figure below for each assay's recommended standard range. Dissolve one vial of Standard in 1 ml of Sample/Standard Dilution Buffer (Solution B) and keep it as a standard stock. Then serially dilute it with Solution B. For example, mix 250 µl of the stock solution with an equal volume of Solution B to make the second stock solution, and then repeat it five more times. The remaining stock solution can be stored at -20°C for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



IgG/IgG2a/IgG2b:	12.5 ng/ml	6.3 ng/ml	3.1 ng/ml	1.6 ng/ml	0.8 ng/ml	0.4 ng/ml	0.2 ng/ml	Blank
IgM:	100 ng/ml	50 ng/ml	25 ng/ml	12.5 ng/ml	6.3 ng/ml	3.1 ng/ml	1.6 ng/ml	Blank

3. **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 (1:100 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 antibodies against Der p2 are observed in normal serum at a 1:100 dilution. If serum samples require a lower dilution than 1:100, please contact support@chondrex.com.

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

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 Secondary Antibody**: Dilute one vial of Secondary Antibody in 10 ml Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

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Strip #	2nd Antibody (µl)	Solution C (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-Der p2 antibodies.

4. The antibody concentration in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration in original test samples.

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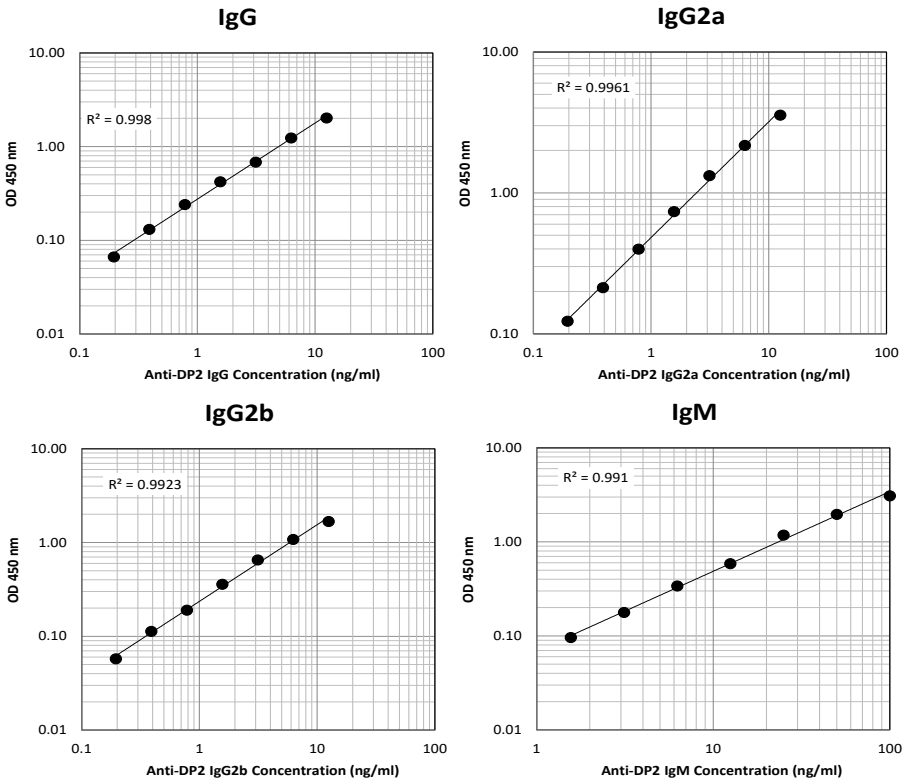
Figure 1 - Typical Standard Curves for the Anti-Der p2 Antibody ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-Der p2 IgG Antibody ELISA Kit

Test	0.4 ng/ml	1.6 ng/ml	6.3 ng/ml
Intra-Assay CV (%)	4.9	4.6	1.3
Inter-Assay CV (%)	6.8	7.7	2.8
Spike Test* (%)	104%	94%	94%

Table 2 - Reproducibility Data for the Mouse Anti-Der p2 IgG2a Antibody ELISA Kit

Test	0.4 ng/ml	1.6 ng/ml	6.3 ng/ml
Intra-Assay CV (%)	4.1	1.2	3.6
Inter-Assay CV (%)	10.1	2.4	8.4
Spike Test* (%)	100%	96%	99%

Table 3 - Reproducibility Data for the Mouse Anti-Der p2 IgG2b Antibody ELISA Kit

Test	0.4 ng/ml	1.6 ng/ml	6.3 ng/ml
Intra-Assay CV (%)	5.1	4.7	5.0
Inter-Assay CV (%)	5.5	5.8	5.7
Spike Test* (%)	95%	92%	93%

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Table 4 - Reproducibility Data for the Mouse Anti-Der p2 IgM Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	1.1	0.9	2.9
Inter-Assay CV (%)	6.3	6.1	3.3
Spike Test* (%)	110%	106%	109%

*Known amounts of anti-Der p2 antibodies were added to samples and then diluted with Sample/Standard Dilution Buffer to assay anti-Der p2 antibodies by ELISA.

TROUBLESHOOTING

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

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