# Mouse Anti-Der p1 Antibody Subtype/Subclass ELISA Kits

Catalog # 3047, 3048, 3049, and 3064

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

## PRODUCT SPECIFICATIONS

| | |
|---|---|
| DESCRIPTION: | ELISA kits to quantify mouse anti-Der p1 antibodies |
| FORMAT: | Precoated 96-well ELISA Plate with removeable strips |
| ASSAY TYPE: | Indirect ELISA |
| ASSAY TIME: | 4.5 hours |
| STANDARD RANGE: | 3047 (IgG) : 100 - 1.6 ng/ml |
| | 3048 (IgG1) : 100 - 1.6 ng/ml |
| | 3049 (IgM) : 250 - 4 ng/ml |
| | 3064 (IgG3) : 20 - 0.3 µg/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: | 3047: Intra-Assay (3-6%)/Inter-Assay (4.9-7.3%)/Spiking Test (93-103%) |
| | 3048: Intra-Assay (1.7-7.3%)/Inter-Assay (6.5-10.6%)/Spiking Test (99-106%) |
| | 3049: Intra-Assay (2.7-3.1%)/Inter-Assay (6.5-10.4%)/Spiking Test (108-114%) |
| | 3064: Intra-Assay (1.7-8.4%)/Inter-Assay (3.8-9.3%)/Spiking Test (102-119%) |
| 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 p1 is a major house dust mite allergen to which more than 70% of patients show an IgE reaction (8).

Der p1 is a cysteine protease consisting of a proenzyme region (80 amino acids) and a mature enzyme region (222 amino acids) (9). In mice, Der p1 has been utilized as a potential candidate for immune tolerance therapies (10) and allergy vaccine development (11), especially for DNA vaccines (12)

To study the immune response to allergens and allergen-specific pathological effects in mouse asthma models, Chondrex, Inc. provides the mouse anti-Der p1 antibody ELISA kits listed below. Chondrex, Inc. also offers ELISA kits for assaying 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: Other antibody subtype ELISA kits against Der p1 as well as Der p1 antigen detection kits are currently under development. Please contact Chondrex, Inc. (support@chondrex.com) for more information.

## LIST OF MOUSE ANTI-DER P1 ANTIBODY SUBTYPE/SUBCLASS ELISA KITS

| Kit | Catalog # |
|---|---|
| Mouse Anti-Der p1 IgG Antibody ELISA Kit | 3047 |
| Mouse Anti-Der p1 IgG1 Antibody ELISA Kit | 3048 |
| Mouse Anti-Der p1 IgM Antibody ELISA Kit | 3049 |
| Mouse Anti-Der p1 IgG3 Antibody ELISA Kit | 3064 |



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## KIT COMPONENTS

| Item | | Quantity | Amount | Storage |
|---|---|---|---|---|
| Standard | IgG (30471) - 100 ng<br>IgG1 (30481) - 100 ng<br>IgM (30491) - 250 ng<br>IgG3 (30641) - 20 µg | 1 vial | Lyophilized | -20°C |
| Secondary Antibody | IgG (30113)<br>IgG1 (30483)<br>IgM (30493)<br>IgG3 (30393) | 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 p1 Extract from *Dermatophagoides pteronyssinus* coated ELISA Plate | | 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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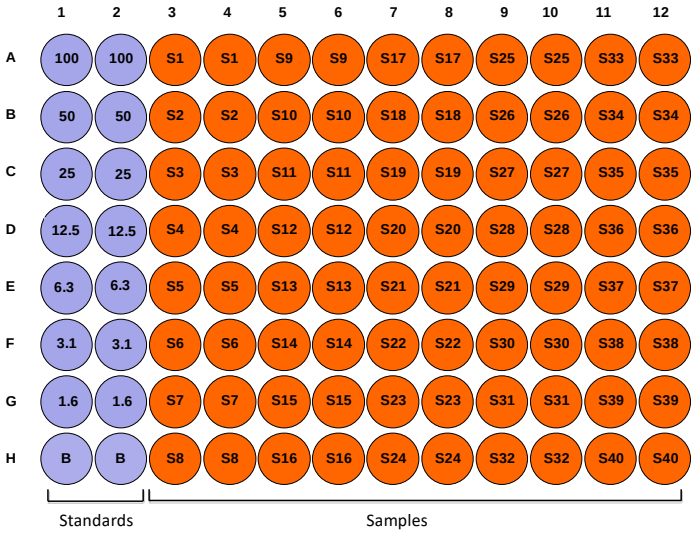
## PLATE MAPPING

Example of the Mouse Anti-Der p1 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.

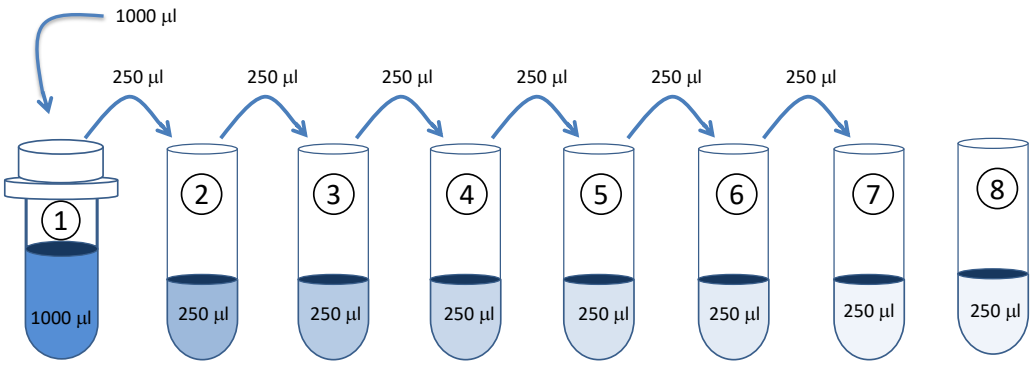
NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -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.

## 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/IgG1: | 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 |
| IgM: | 250 ng/ml | 125 ng/ml | 63 ng/ml | 31 ng/ml | 16 ng/ml | 8 ng/ml | 4 ng/ml | Blank |
| IgG3: | 20 µg/ml | 10 µg/ml | 5 µg/ml | 2.5 µg/ml | 1.25 µg/ml | 0.63 µg/ml | 0.31 µg/ml | Blank |

3. **Prepare Sample Dilutions**: The dilution of mouse serum immunized with Der p1 or HDM will vary (1:100 or more) depending on the immunization schedule and timing of serum collection. In general, no antibodies against Der p1 are observed in normal serum at a 1:100 dilution.

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.

| 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

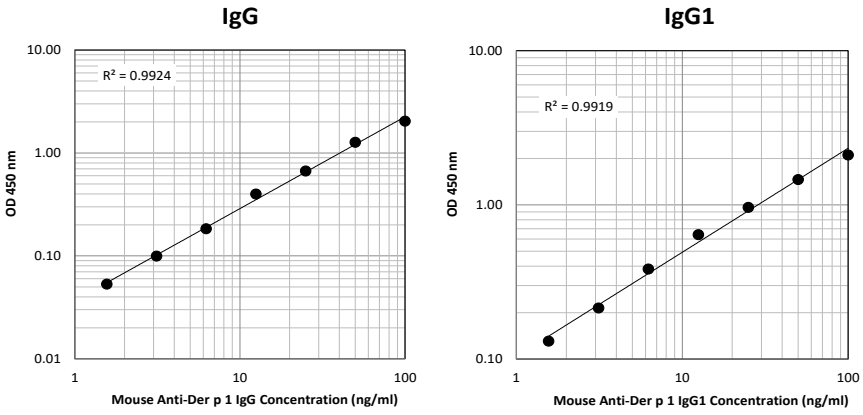
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 p1 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.

Figure 1 - Typical Standard Curves for the Anti-Der p1 Antibody ELISA Kits



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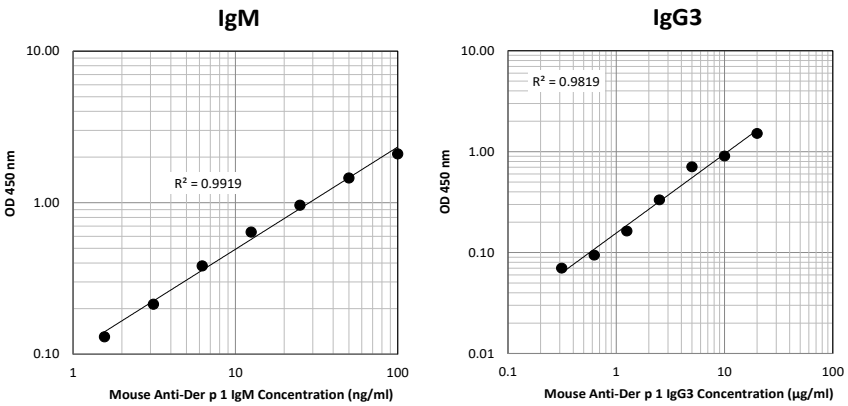


## VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-Der p1 IgG Antibody ELISA Kit

| Test | 3.2 ng/ml | 12.5 ng/ml | 50 ng/ml |
|---|---|---|---|
| Intra-Assay CV (%) | 6.0 | 3.0 | 5.6 |
| Inter-Assay CV (%) | 4.9 | 7.1 | 7.3 |
| Spike Test* (%) | 103% | 102% | 93% |

Table 2 - Reproducibility Data for the Mouse Anti-Der p1 IgG1 Antibody ELISA Kit

| Test | 3.2 ng/ml | 12.5 ng/ml | 50 ng/ml |
|---|---|---|---|
| Intra-Assay CV (%) | 6.6 | 1.7 | 7.3 |
| Inter-Assay CV (%) | 8.0 | 6.5 | 10.5 |
| Spike Test* (%) | 99% | 101% | 106% |

Table 3 - Reproducibility Data for the Mouse Anti-Der p1 IgM Antibody ELISA Kit

| Test | 8 ng/ml | 31 ng/ml | 125 ng/ml |
|---|---|---|---|
| Intra-Assay CV (%) | 3.1 | 2.7 | 3.0 |
| Inter-Assay CV (%) | 6.5 | 10.4 | 9.0 |
| Spike Test* (%) | 108% | 114% | 111% |

Table 4 - Reproducibility Data for the Mouse Anti-Der p1 IgG3 Antibody ELISA Kit

| Test | 0.63 µg/ml | 2.5 µg/ml | 10 µg/ml |
|---|---|---|---|
| Intra-Assay CV (%) | 3.2 | 1.7 | 8.4 |
| Inter-Assay CV (%) | 9.3 | 3.8 | 7.8 |
| Spike Test* (%) | 104% | 102% | 119% |

*Known amounts of anti-Der p1 antibodies were added to samples and then diluted with Sample/Standard Dilution Buffer to assay anti-Der p1 antibodies by ELISA.



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## TROUBLESHOOTING

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

## REFERENCES

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