House Dust Mite Der p 1 Detection ELISA Kit

Catalog # 6044

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify house dust mite Der p 1
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	10 - 0.16 µg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Culture media, serum, plasma, and solubilized samples (extracts)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (1.8-6.5%)/Inter-Assay (6.3-8.9%)/Spiking Test (94-111%)
NOTES:	N/A

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INTRODUCTION

Allergic diseases and symptoms arise from an active immune response to antigens which are usually harmless, such as pollen, pet dander, or food. 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). Detecting indoor allergens can be helpful in controlling environmental factors that exacerbate asthma. In fact, higher levels of Der p1 allergen are associated with greater asthma severity, highlighting the role of Der p1 as an independent risk factor for asthma severity (10).

To analyze the pathogenesis of allergens in allergic reactions in patients or animal disease models, Chondrex, Inc. provides a HDM Der p1 Detection ELISA Kit. Chondrex, Inc. also offers ELISA kits for assaying other allergens, such as House Dust Mite (Der p10), Gliadin, Ovalbumin, Peanut Ara h2, and Peanut Ara h6. Please visit www.chondrex.com for more information.

NOTE: This kit cross-reacts with the Group 1 allergen from *Dermatophagoides farinae* (Der f1) by approximately less than 10%.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard (60441)	1 vial	10 µg, Lyophilized	-20°C
Detection Antibody (60443)	1 vial	100 µl	-20°C
Solution B - Sample/Standard Dilution Buffer (30055)	1 Bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	2 Bottles	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB (90023)	2 vials	200 µl	-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
Capture Antibody Coated 96-Well ELISA Plate (Blue)	1 each	8-well Strips x12	-20°C

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ASSAY OUTLINE

Add 100 µl of diluted Standards and Samples

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

Add 100 µl of diluted Detection Antibody solution

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

Add 100 µl of diluted Streptavidin Peroxidase solution

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

Add 100 µl of TMB solution

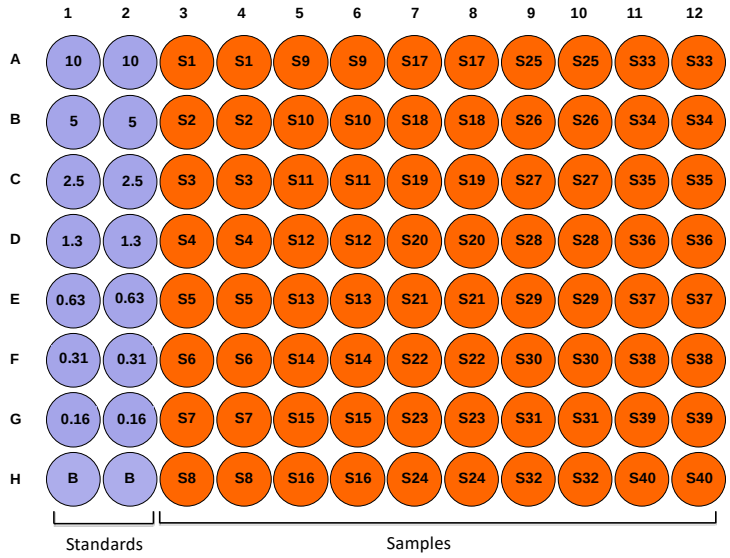
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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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.

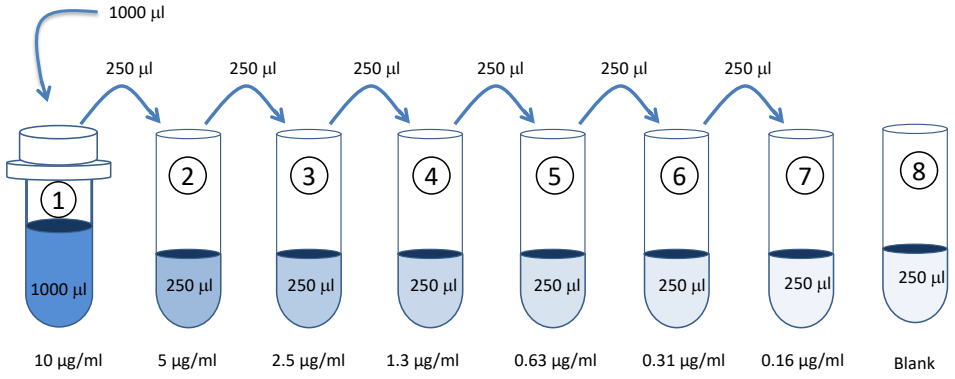
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.16 - 10 µg/ml. Dissolve one vial of Der p1 standard with 1 ml of Sample/Standard Dilution Buffer (Solution B) for the 10 µg/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (10 µg/ml) with an equal volume of Solution B to make a 5 µg/ml solution, and then repeat it five more times for 2.5, 1.25, 0.63, 0.31, and 0.16 µg/ml solutions. The remaining 10 µg/ml standard stock may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



2. **Prepare Samples:** Dilute samples at least 1:1 with Solution B depending on the estimated Der p1 level in the samples. Two to three different sample dilutions are recommended if the Der p1 levels in the samples are unknown.

   NOTE: Samples must be diluted with Solution B to maintain optimal assay conditions.

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

4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X 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 Detection Antibody Solution**: Prepare the detection antibody solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution D (ml)
2	17	1.7
4	25	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 1 hour.

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**: Dilute one vial of TMB in 10 ml of 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.

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.

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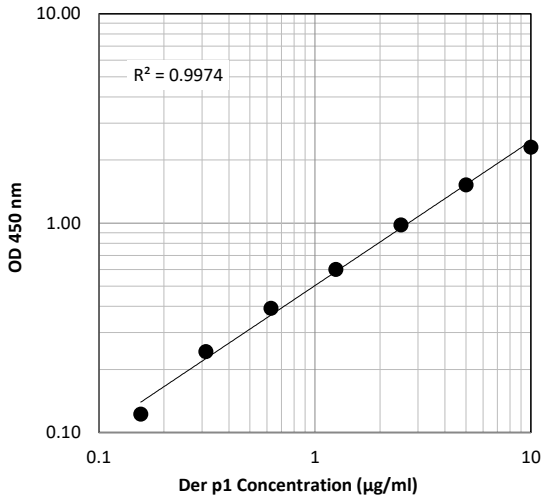
CALCULATING RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the “blank” (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of Der p1 (µg/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 0.16 - 10 µg/ml.
4. The µg/ml of Der p 1 in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the Der p 1 Detection ELISA Kit



ASSAY VALIDATION

Table 1 – Reproducibility Data for the Der p1 Detection ELISA Kit

Test	0.31 µg/ml	1.25 µg/ml	5 µg/ml
Intra-Assay CV (%)	1.8	6.5	3.1
Inter-Assay CV (%)	6.3	8.9	7.3
Spike Test* (%)	94%	99%	111%

* Known amounts of Der p1 were added to samples and diluted with Sample/Standard Dilution Buffer (Solution B).

TROUBLESHOOTING

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

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