House Dust Mite (Der p 10 and Der f 10) Detection ELISA Kit

Catalog # 6031

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify house dust mite (Der p 10 and Der f 10)
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	4000 - 62.5 pg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	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 (3.8-6.2%)/Inter-Assay (2.4-8.9%)/Spiking Test (93-105%)
NOTES:	N/A

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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INTRODUCTION

Allergic diseases and symptoms result from an active immune response to typically harmless antigens, such as pollen, pet dander, or food. Asthma is a common chronic inflammatory disease that affects 300 million people worldwide, across all age groups (1, 2). It is triggered by exposure to allergens such as dust mites, pet dander, pollen, or mold and is characterized by airflow obstruction and bronchospasms. Among asthma allergens, house dust mite (HDM) is the most prevalent, affecting up to 85% of asthma patients (3, 4).

The two main HDM species, *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f), produce more than 20 allergens, classified based on sequence and functional similarities. Among these, group 1 (Der 1) and group 2 (Der 2) allergens dominate allergic responses and are the most extensively studied (5–7). Der p 1, in particular, is a major allergen to which over 70% of patients exhibit an IgE reaction (8).

Another allergen, group 10 allergen (Der p 10), has gained attention for its role as a cross-reactive allergen found in invertebrates, such as seafood, where it is implicated in severe systemic anaphylaxis (9). Der p 10 is composed of 284 amino acids, with a predicted molecular mass of 32.9 kDa and an isoelectric point of 4.78. It shares a high degree of sequence homology (95%) with Der f 10 (10). The monoclonal antibodies in this kit are capable of detecting both Der p 10 and Der f 10 with equal efficiency.

Approximately 15.2% of HDM-allergic patients are sensitized to Der p 10. Patients who are Der p 10-negative are primarily sensitized to major allergens like Der p 1 and Der p 2. Conversely, Der p 10-positive patients tend to react to multiple HDM allergens or exhibit selective reactivity to Der p 10. Although the allergenic activity of Der p 10 is generally low, some patients with clinically significant HDM allergies are sensitized to this allergen (11).

To advance research on allergen pathogenesis in both patients and animal models, Chondrex, Inc. has recently improved its HDM (Der p 10 and Der f 10) Detection ELISA Kit, which now offers a 50-fold increase in assay sensitivity. In addition, Chondrex, Inc. provides ELISA kits for the analysis of other allergens, including HDM (Der p 1), gluten allergens (gliadin and glutenin), ovalbumin, and peanut allergens (Ara h 1, Ara h 2, Ara h 3, and Ara h 6). For more information, please visit www.chondrex.com or contact support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Recombinant Der f 10 Standard (60314)	1 vial	4000 pg, Lyophilized	-20°C
Detection Antibody (60313)	1 vial	100 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 Bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 Bottle	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 (Gray)	1 each	8-well Strips x12	-20°C

ASSAY OUTLINE

Add 100 µl of diluted standards and samples into wells

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

Add 100 µl of diluted Detection Antibody

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

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
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

	1	2	3	4	5	6	7	8	9	10	11	12
A	4000	4000	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	2000	2000	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	1000	1000	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	500	500	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	250	250	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	125	125	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	63	63	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

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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.

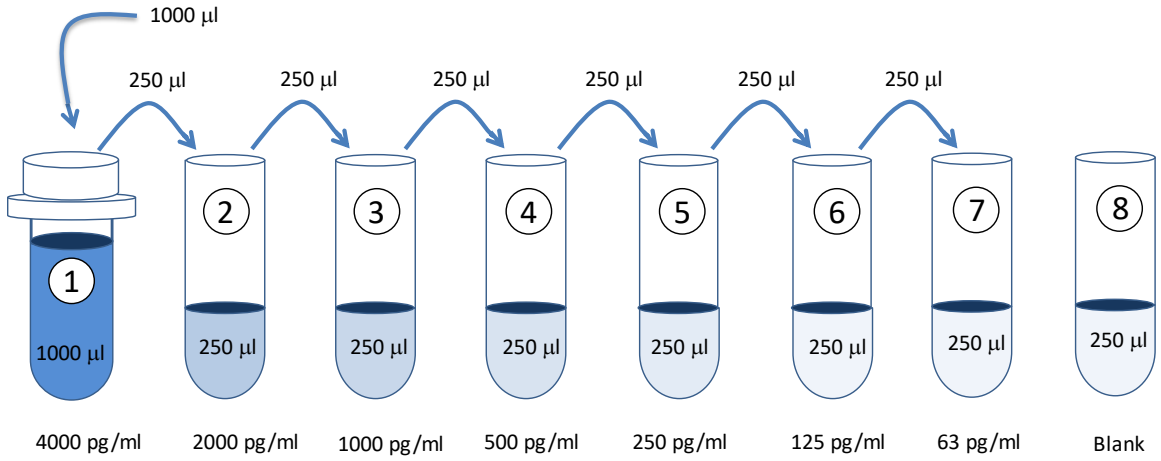
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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 62.5 - 4000 pg/ml. Dissolve one vial of standard with 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 4000 pg/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (4000 pg/ml) with an equal volume of Solution B to make a 2000 pg/ml solution, and then repeat it five more times for 1000, 500, 250, 125, and 62.5 pg/ml solutions. The remaining 4000 pg/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 HDM (Der p 10 and Der f 10) level in the samples. Two to three different sample dilutions are recommended if the HDM (Der p 10 and Der f 10) 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 Sample/Standard/Detection Antibody Dilution Buffer (Solution B) 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 B (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**: 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

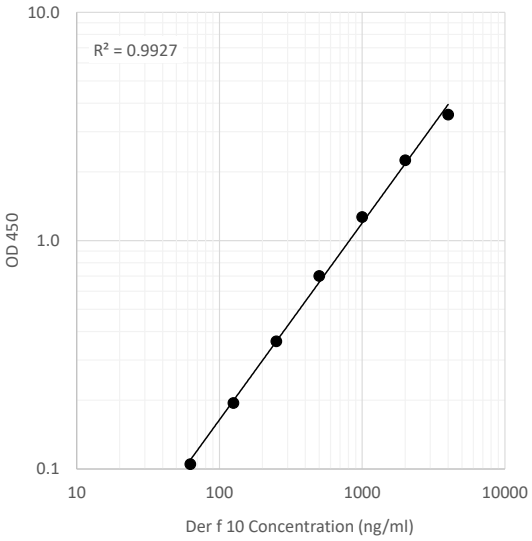
10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

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.

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 f 10 (pg/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 62.5 - 4000 pg/ml.
4. The pg/ml of HDM (Der p 10 and Der f 10) in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the HDM (Der p 10 and Der f 10) Detection ELISA Kit



ASSAY VALIDATION

Table 1 – Reproducibility Data for the HDM (Der p 10 and Der f 10) Detection ELISA Kit

Test	125 pg/ml	500 pg/ml	2000 pg/ml
Intra-Assay CV (%)	5.4	3.8	6.2
Inter-Assay CV (%)	8.9	4.4	2.4
Spike Test* (%)	96%	105%	93%

* Known amounts of HDM (Der p 10 and Der f 10) were added to samples and diluted with Sample/Standard/Detection Antibody 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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