Acarus siro Antigen Detection ELISA Kit

Catalog # 6050

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify *Acarus siro* antigens (grain mite)
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	250 - 4 ng/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 (2.3-5.8%)/Inter-Assay (2.9-3.9%)/Spiking Test (95-98%)
NOTES:	N/A

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INTRODUCTION

Asthma and other respiratory diseases caused by sensitization to mites are global health problems. Because mites are found in both temperate and subtropical climates, a large proportion of the world's population is exposed. In addition to the well-documented mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae,* species such as *Acarus siro* and *Lepidoglyphus destructor* are emerging as significant contributors to allergies also (1). These storage dust mites (SDM) are commonly found in stored foods such as grains, flour, and hay and they can thrive in warm, humid conditions with an optimum relative humidity of 80% and temperatures between 25 and 30°C (2). They are known to cause symptoms such as bronchial asthma, allergic rhinitis, and conjunctivitis, particularly in rural work environments (3,4). Oral mite anaphylaxis (also known as Pancake Syndrome) is a relatively new condition where patients develop moderate-to-severe allergic symptoms after consuming mite-contaminated flour-based foods (5).

There are few studies regarding the detection of *Acarus siro*, even though it is a significant and widespread pest of foods such as soybeans, wheat, and almonds (6). *Acarus siro* mites shed feces, eggs, microorganisms, etc. into stored foods and these things can lead to allergic symptoms, especially if the food is consumed raw or undercooked (7). Depending on the food, this may lead to misdiagnoses of wheat or gluten allergies (8). While complete elimination of *Acarus siro* mites in stored foods is impossible, identifying and controlling systemic infestation is important, so early detection is necessary (7).

Currently, there is little research available investigating the role of *Acarus siro* in allergies. To accommodate this, Chondrex, Inc. provides an *Acarus siro* Antigen Detection ELISA Kit (Cat # 6050). This kit can be used to assay food products and storage conditions to determine the levels of *Acarus siro* mite extract present. Chondrex, Inc. also offers ELISA kits for assaying Mouse Anti-*Acarus siro* antibody subtypes/subclasses as well as other mite and mite-allergen antibody subtypes/subclasses and total immunoglobulin subtypes/subclasses. Please visit www.chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard (60501)	1 vial	250 ng, Lyophilized	-20°C
Detection Antibody (60503)	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 (Brown)	1 each	8-well Strips x12	-20°C

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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

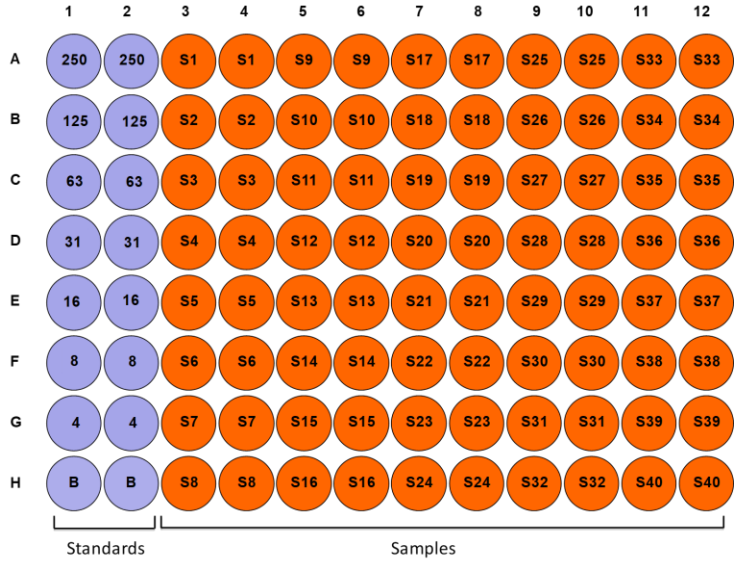
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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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.

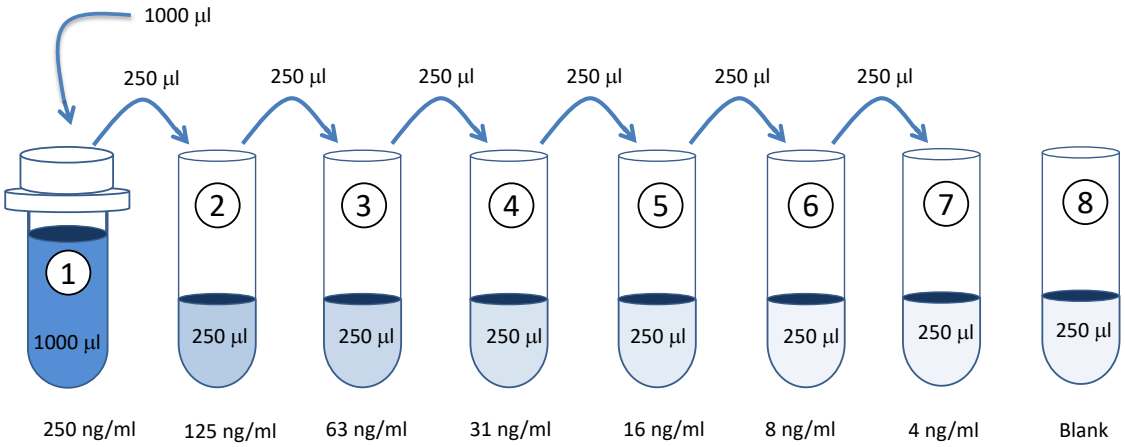
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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 4 – 250 ng/ml. Dissolve one vial of standard with 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 250 ng/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (250 ng/ml) with an equal volume of Solution B to make a 125 ng/ml solution, and then repeat it five more times for 63, 31,16, 8, and 4 ng/ml solutions. The remaining 250 ng/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 *Acarus siro* level in the samples. Two to three different sample dilutions are recommended if the *Acarus siro* 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.

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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**: Use new tubes when preparing 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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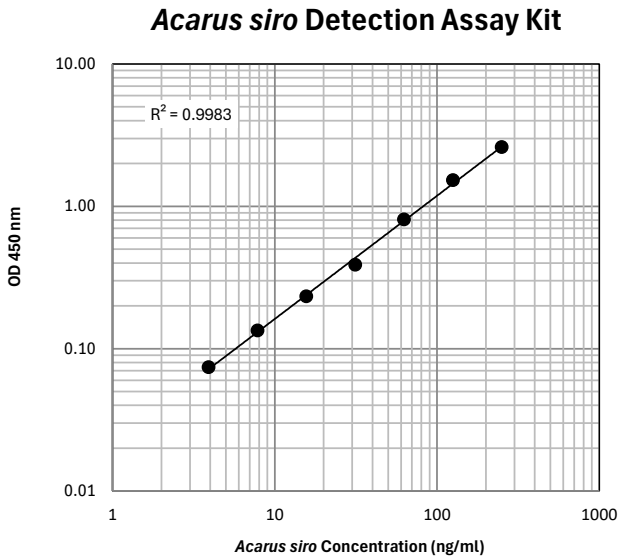
CALCULATING RESULTS

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 *Acarus siro* (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 4 – 250 ng/ml.
4. The ng/ml of *Acarus siro* in test samples can be calculated using regression analysis.

Figure 1 – A Typical Standard Curve for the *Acarus siro* Detection ELISA Kit



ASSAY VALIDATION

Table 1 – Reproducibility Data for the *Acarus siro* Detection ELISA Kit

Test	7.8 ng/ml	31 ng/ml	125 ng/ml
Intra-Assay CV (%)	4.3	5.8	2.3
Inter-Assay CV (%)	3.6	2.9	3.9
Spike Test* (%)	96%	98%	95%

* Known amounts of *Acarus siro* 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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