Peanut Ara h6 Detection ELISA Kit

Catalog # 6042

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify Ara h6
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	2000 - 31 pg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Liquid samples and biological fluids (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (4.5-9.0%)/Inter-Assay (3.0-3.5%)/Spiking Test (101-104%)
NOTES:	Ara h6 in samples must be extracted before starting assays

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INTRODUCTION

Immediate hypersensitivity reactions to peanuts, an IgE-mediated food allergy, have been a major public health concern for many years, particularly in westernized countries where peanut allergies can persist into adulthood. For allergic patients, avoidance currently remains the only viable option (1).

Eleven potentially important peanut allergens have been identified. Ara h1, Ara h2, Ara h3, and Ara h6 have been designated the major peanut allergens. Ara h2 and Ara h6, two highly related 2S albumins belonging to seed storage proteins, especially contribute to the development of allergic reactions (2). Ara h2 contains ten independent IgE-binding linear epitopes. Two isoforms, Ara h 2.01 and Ara h 2.02, were identified respectively as a 17 and 19-kDa doublet on SDS-PAGE. The larger isoform containing twelve extra amino acids including a duplication of a strong IgE-binding sequence, DPYSPS, can bind more IgE antibodies (3, 4). Ara h6 has a molecular weight of 14.5 kDa and is 59 % homologous to Ara h2. It also possesses heat and digestion stability, a protease-stable core, and allergenic potency similar to that of Ara h2 (5-6).

The methods used to analyze allergens in foods are often divided into either DNA-based methods or protein-based methods. Immunoassays are one example of protein-based methods, and they are used in many industries for detecting allergens in foods. Enzyme-linked immunosorbent assays (ELISA) are commonly used for validating contamination of allergens in food products because of their high-precision, easy handling, and good potential for standardization (7).

As a peanut allergen, Ara h6 can be a useful analytical target for validating peanut-free foods. This application is not limited to only food testing either, as interestingly, serum Ara h6 levels correlate with peanut allergy tolerance in patients and can be a useful marker of tolerance levels in therapeutic studies (8).

Chondrex, Inc provides an Ara h6 detection ELISA kit for analyzing food quality as well as for studying the distribution and dynamics of Ara h6. Please visit www.chondrex.com or contact support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Ara h6 (60421)	1 vial	2000 pg, lyophilized	-20°C
Anti-Ara h6 Detection Antibody (60423)	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)	2 bottles	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-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
Anti-Ara h6 Antibody Coated ELISA Plate (Red)	1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

Add 100 µl of diluted standards or samples in wells

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

Add 100 µl of diluted Detection Antibody

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

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plates.

Add 100 µl of TMB solution

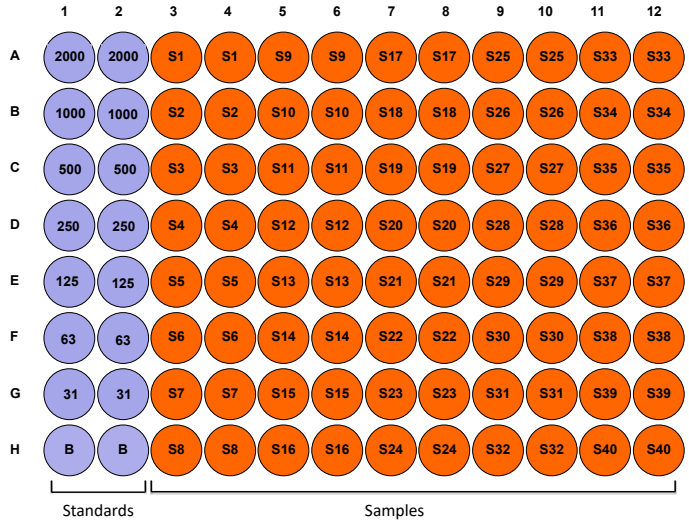
↓ Incubate at room temperature for 25 minutes.

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.

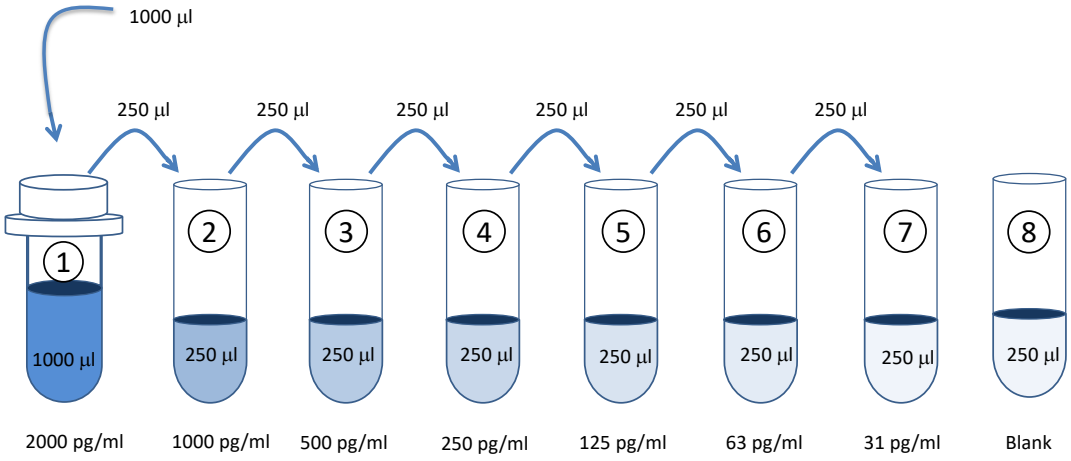
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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 31 - 2000 pg/ml. Dissolve one vial of Standard (2000 pg/vial) in 1 ml of Sample/Standard/Detection Antibody 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 2000 pg/ml solution with an equal volume of Solution B to make a 1000 pg/ml solution, and then repeat it five more times for 500, 250, 125, 63, and 31 pg/ml standard solutions.



2. **Prepare Sample Dilutions**: Dilute samples at least 1:1 with Solution B depending on the estimated Ara h6 level in the samples. Two or three different sample dilutions are recommended if the Ara h6 levels in the samples are unknown.

3. **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.

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.*

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5. **Add Ara h6 Detection Antibody**: Dilute one vial of Anti-Ara h6 Detection Antibody in 10 ml Streptavidin Peroxidase Dilution Buffer (Solution D). 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	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. 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.

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CALCULATING RESULTS

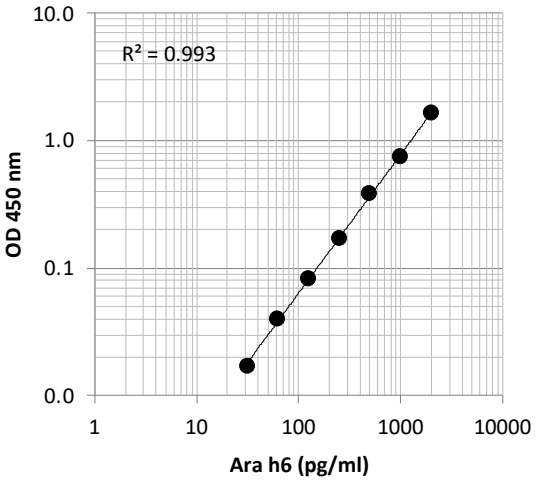
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 pg/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 31 to 2000 pg/ml.

4. The pg/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the Ara h6 concentration (pg/ml) in the original test samples.

Figure 1 - A Typical Standard Curve for the Ara h6 Detection ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility Data for the Ara h6 Detection ELISA Kit

Test	60 pg/ml	250 pg/ml	1000 pg/ml
Intra-Assay CV (%)	5.2	4.5	9.0
Inter-Assay CV (%)	3.5	3.0	3.0
Spike Test* (%)	101%	104%	102%

*Known amounts of Ara h6 were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay Ara h6 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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