Mouse Anti-Peanut Allergen IgE Antibody ELISA Kits

Catalog # 3063, 3071, 3106, and 3107

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify mouse IgE antibodies against crude peanut extract (CPE), Ara h 1, Ara h 2, or Ara h 3.
	3063: Mouse Anti-Crude Peanut Extract (CPE) IgE Antibody ELISA Kit
	3107: Mouse Anti-Ara h 1 (Peanut Allergen) IgE Antibody ELISA Kit
	3106: Mouse Anti-Ara h 2 (Peanut Allergen) IgE Antibody ELISA Kit
	3071: Mouse Anti-Ara h 3 (Peanut Allergen) IgE Antibody ELISA Kit
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5 hours
STANDARD RANGE:	3063, 3017, and 3107: 50 – 0.8 ng/ml
	3106: 400 – 6.3 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3063: Intra-Assay (1.1-8.8%)/Inter-Assay (1.2-7.4%)/Spiking Test (92-94%)
	3107: Intra-Assay (2.8-4.5%)/Inter-Assay (3.1-6.3%)/Spiking Test (93-105%)
	3106: Intra-Assay (3.3-6.6 %)/Inter-Assay (1.5-4.9%)/Spiking Test (93-105%)
	3071: Intra-Assay (1.3-4.5%)/Inter-Assay (1.0-4.1%)/Spiking Test (93-106%)
NOTES:	N/A

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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 h 1, Ara h 2, Ara h 3, and Ara h 6 have been designated the major peanut allergens. Ara h 2 and Ara h 6, two highly related 2S albumins, especially contribute to the development of allergic reactions (2). Mouse peanut allergy models have been used to study the pathogenesis of the peanut allergy and to help develop new treatments. The mouse models can be induced by administration of crude peanut extract (CPE) or each purified Ara allergen and evaluated for the humoral immune responses such as serum anti-IgE and IgG antibodies against the allergen, T-cell mediated immune response associated cytokines levels, as well as body temperature and clinical signs of anaphylaxis. These factor changes observed in the disease models are useful for studying the efficacy of protective effects against the development of allergic reactions (3–9). Additionally, the choice of mouse strain may be important, as reactivity to peanut allergens may vary depending on the genetic background. For example, specific IgE responses were directed against Ara h 3 in C3H, C57BL/6, and BALB/c mice. Meanwhile, anti-Ara h 1 and Ara h 2 IgE antibodies were found to be higher in C3H mice than in other strains (10, 11).

To evaluate the humoral immune response against CPE in mouse allergy models, Chondrex, Inc. provides ELISA kits for assaying mouse IgE antibodies against CPE (Cat # 3063), Ara h 1 (Cat # 3107), Ara h 2 (Cat # 3106), and Ara h 3 (Cat # 3071). Chondrex, Inc. also offers ELISA kits for assaying anti-CPE, ovalbumin, house dust mite, and gliadin antibody subtypes/subclasses as well as total immunoglobulin subtypes/subclasses. For more information, please visit www.chondrex.com or contact support@chondrex.com.

LIST OF MOUSE ANTI-PEANUT ANTIBODY SUBTYPE/SUBCLASS ELISA KITS

Mouse Anti-Peanut Allergen Antibody ELISA Kit	Catalog #			
	CPE	Ara h 1	Ara h 2	Ara h 3
IgG	3056	3074	3077	3082
IgG1	3057	3084	Coming Soon!	3083
IgG2b	3059	3086	3078	Coming soon!
IgG2a	3058	3088	Coming soon!	Coming soon!
IgG3	3060	Coming Soon!	Coming soon!	Coming soon!
IgA	3061	Coming Soon!	3080	Coming soon!
IgE	3063	3107	3106	3071
IgM	3062	Coming Soon!	3079	Coming soon!

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KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse Anti-CPE IgE Antibody (30631) Standard Mouse Anti-Ara h 1 IgE Antibody (31071) Standard Mouse Anti-Ara h 2 IgE Antibody (31061)	1 vial	50 ng, lyophilized 50 ng, lyophilized 400 ng, lyophilized	-20°C
Biotinylated CPE (30633) Biotinylated Ara h 1 (31073) Biotinylated Ara h 2 (31063) Biotinylated Ara h 3 (30713)	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 Concentrate (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-Mouse IgE Antibody Coated ELISA Plate (Yellow)	1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE

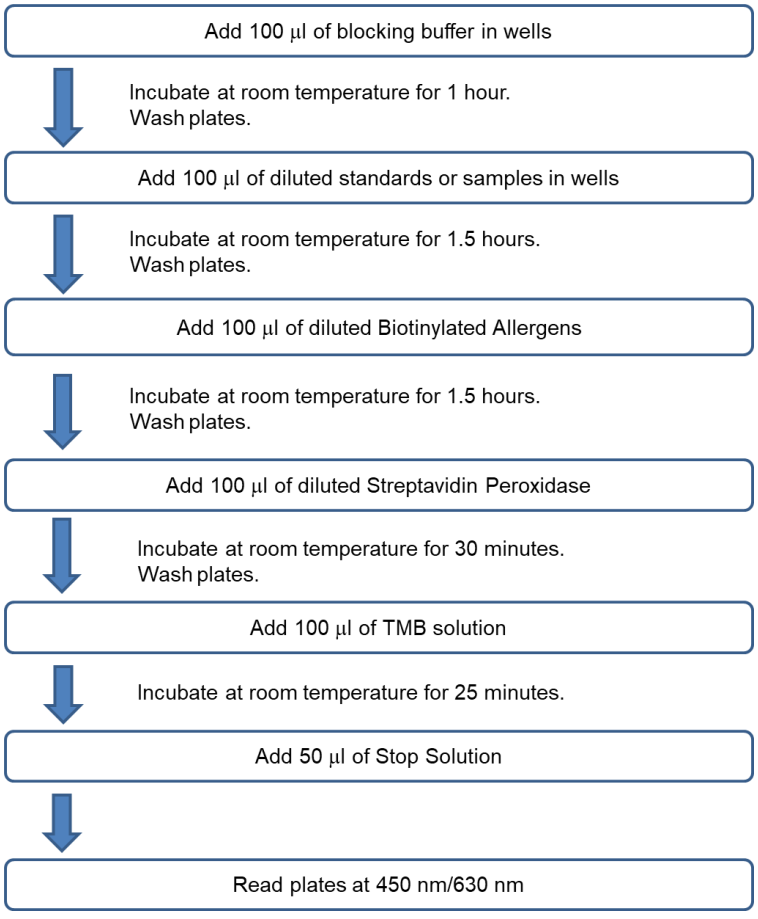

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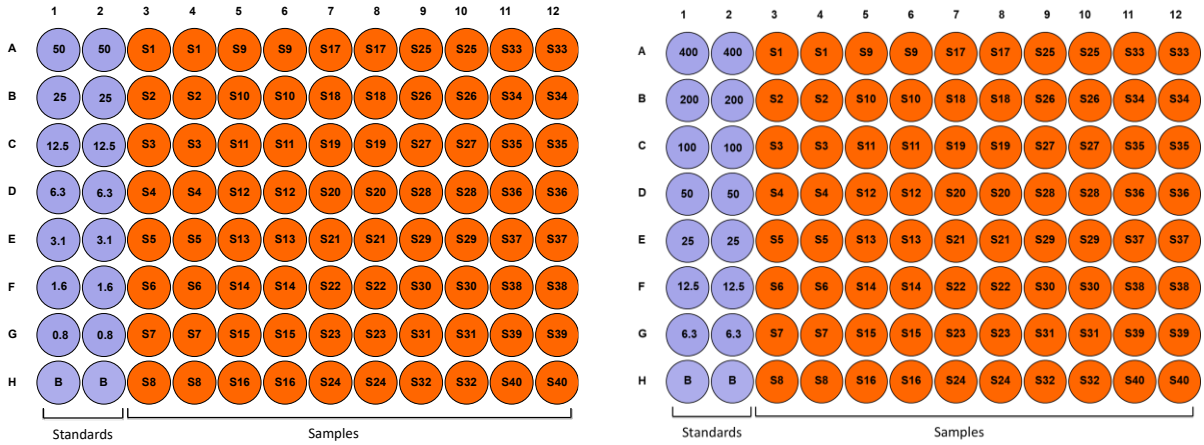
PLATE MAPPING

Examples of the Mouse Anti-CPE, Ara h 1 IgE, and Ara h 3 IgE Antibody ELISAs (Left) and Ara h 2 IgE Antibody ELISA (Right)



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: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

NOTE 7: Serum IgE antibodies are a mixture of multiple antibodies with a variety of affinity ranges. The OD value obtained in ELISA for an antibody assay depends on the antibody concentration as well as the antibody affinity towards an antigen. In general, ELISA data using a monoclonal antibody as a standard does not reflect the absolute levels of IgE. Therefore, IgE levels determined using this kit should be expressed as ng of IgE per ml.

NOTE 8: If the total IgE concentration in a sample is higher than 500 ng/ml, the sample must be diluted to lower the total IgE levels below 500 ng/ml because the anti-CPE IgE value obtained from this ELISA may be lower than the actual value due to competition from non-anti-CPE IgE antibodies in sample. Therefore, it is strongly recommended that total IgE levels be determined first using the Mouse Total IgE Assay Kit (Cat # 3005).

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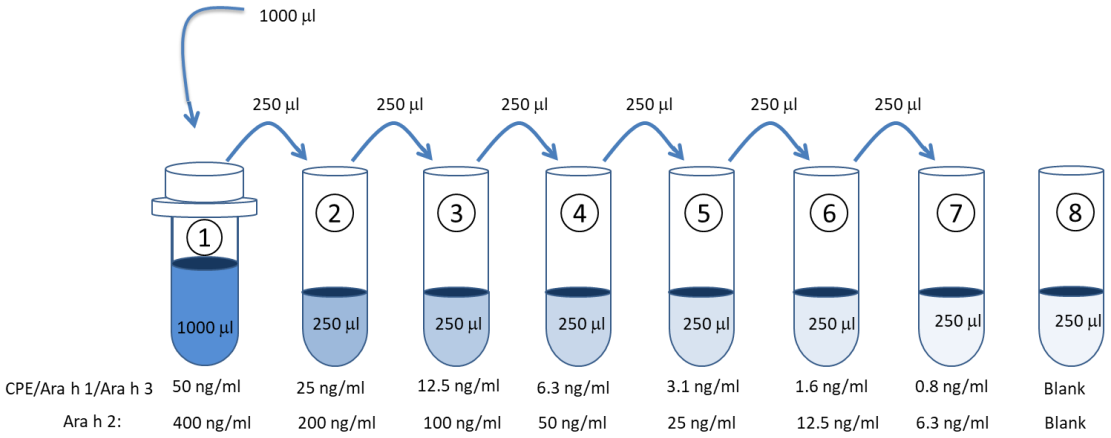
ASSAY PROCEDURE

1. **Add Blocking Buffer:** Add 100 µl of the Blocking 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/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 first stock solution with an equal volume of Solution B to make the second standard solution, and then repeat it five more times. The original standard stock solution can be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



3. **Prepare Sample Dilutions**: An important point to note is that the composition of CPE mixtures can exhibit variations depending on the vendor, batch, and manufacturing process. These variations can result in differing levels of antigens in the final CPE product, which when used to immunize mice, can impact the serum antibody levels against those antigens. The dilution of serum from mice immunized with CPE antigens varies (1:1 or more) depending on the immunization schedule and timing of serum collection. In general, no antibodies against CPE are observed in normal serum at a 1:1 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 1.5 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 Biotinylated Allergen**: Prepare biotinylated CPE, Ara h 1, Ara h 2, **OR** Ara h 3 solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. ***Do not mix different biotinylated allergens in the same tube.*** Add 100 µl of biotinylated allergen solution to each well and incubate at room temperature for 1.5 hours.

Strip #	Biotinylated Allergen (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

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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 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

10. **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.*

11. **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. After adding the required volume of TMB concentrate to the buffer, suspend and vortex the mixture immediately. 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

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

13. **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-CPE IgE, Ara h 1, Ara h 2, and Ara h 3 antibodies.

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

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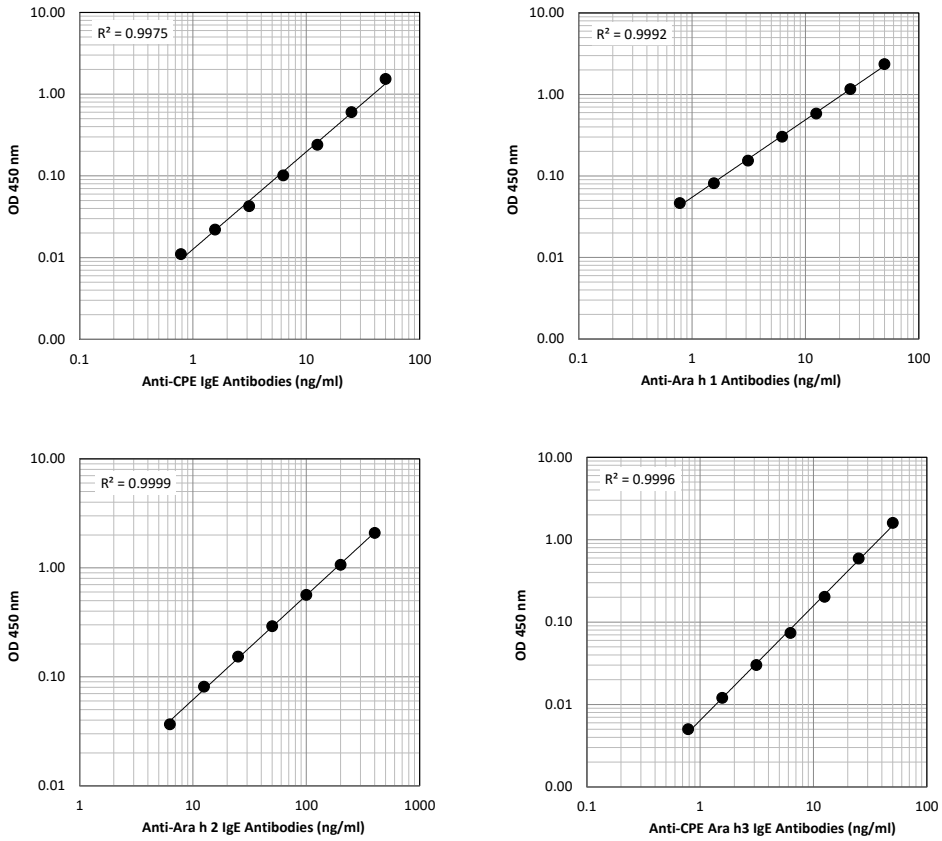
Figure 1 - Typical Standard Curves for the Anti-CPE, Ara h 1, Ara h 2, and Ara h 3 IgE Antibody ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-CPE IgE Antibody ELISA Kit

Test	1.25 ng/ml	5 ng/ml	25 ng/ml
Intra-Assay CV (%)	8.8	6.0	1.1
Inter-Assay CV (%)	7.4	4.1	1.2
Spike Test* (%)	94%	93%	92%

Table 2 - Reproducibility Data for the Mouse Anti-Ara h 1 IgE Antibody ELISA Kit

Test	1.6 ng/ml	6 ng/ml	25 ng/ml
Intra-Assay CV (%)	4.5	3.2	2.8
Inter-Assay CV (%)	6.3	3.1	5.3
Spike Test* (%)	93%	97%	105%

Table 3 - Reproducibility Data for the Mouse Anti-Ara h 2 IgE Antibody ELISA Kit

Test	6.5 ng/ml	30 ng/ml	150 ng/ml
Intra-Assay CV (%)	4.5	3.3	6.6
Inter-Assay CV (%)	1.5	3.7	4.9
Spike Test* (%)	99%	104%	109%

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Table 4 - Reproducibility Data for the Mouse Anti-Ara h 3 IgE Antibody ELISA Kit

Test	2.1 ng/ml	8.3 ng/ml	33.2 ng/ml
Intra-Assay CV (%)	4.5	1.3	2.0
Inter-Assay CV (%)	1.0	2.2	4.1
Spike Test* (%)	106%	93%	94%

*Known amounts of anti-CPE, Ara h 1, Ara h 2 or Ara h 3 IgE antibodies were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay anti-CPE, Ara h 1 IgE, Ara h 2 IgE, or Ara h 3 IgE antibodies 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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