Mouse Anti- Crude Peanut Extract (CPE) IgE Antibody ELISA Kit

Mouse Anti- Ara h3 (Peanut Allergen) IgE Antibody ELISA Kit

Catalog # 3063 and 3071

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify mouse anti-crude peanut extract (CPE) or anti-Ara h3 IgE antibodies
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5 hours
STANDARD RANGE:	50 – 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:10 (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%)
	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 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, 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 mainly directed against Ara h3 in C57BL/6 and BALB/c mice, while Ara h2 was the main target in C3H mice. (10, 11)

To evaluate the humoral immune response against CPE in mouse allergy models, Chondrex, Inc. provides ELISA kits for assaying mouse anti-CPE and anti-CPE Ara h3 IgE antibodies (Catalog # 3063 and 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

Kit	CPE Catalog #	Ara h2 Catalog #	Ara h3 Catalog #
Mouse Anti-Peanut IgG Antibody ELISA Kit	3056	3077	3082
Mouse Anti-Peanut IgG1 Antibody ELISA Kit	3057	Coming Soon!	3083
Mouse Anti-Peanut IgG2b Antibody ELISA Kit	3059	3078	Coming soon!
Mouse Anti-Peanut IgM Antibody ELISA Kit	3062	3079	Coming soon!
Mouse Anti-Peanut IgE Antibody ELISA Kit	3063	Coming soon!	3071
Mouse Anti-Peanut IgG2a Antibody ELISA Kit	3058	Coming soon!	Coming soon!
Mouse Anti-Peanut IgG3 Antibody ELISA Kit	3060	Coming soon!	Coming soon!
Mouse Anti-Peanut IgA Antibody ELISA Kit	3061	3080	Coming soon!

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse Anti-CPE IgE Antibody (30631)	1 vial	50 ng, lyophilized	-20°C
Biotinylated CPE (30633) Biotinylated Ara h3 (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 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-Mouse IgE Antibody Coated ELISA Plate (Yellow)	1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE

Add 100 µl of blocking buffer in wells

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

Add 100 µl of diluted standards or samples in wells

↓ Incubate at room temperature for 1.5 hours.
Wash plates.

Add 100 µl of diluted Biotinylated CPE (3063) or Biotinylated Ara h3 (3071)

↓ Incubate at room temperature for 1.5 hours.
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

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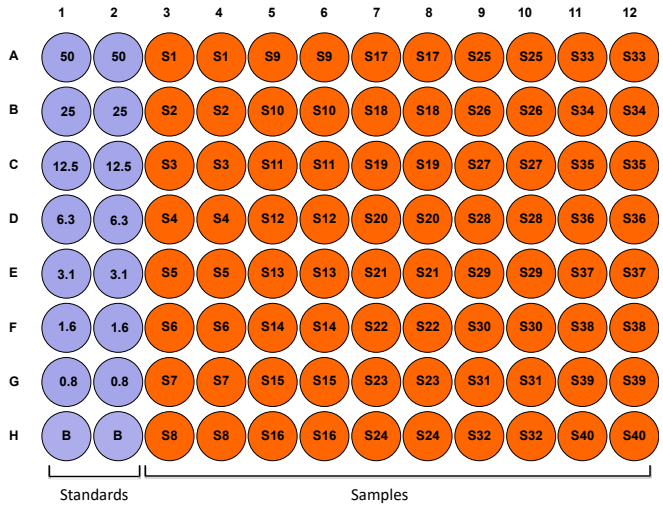
PLATE MAPPING



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 -20°C 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.

NOTE 8: 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.

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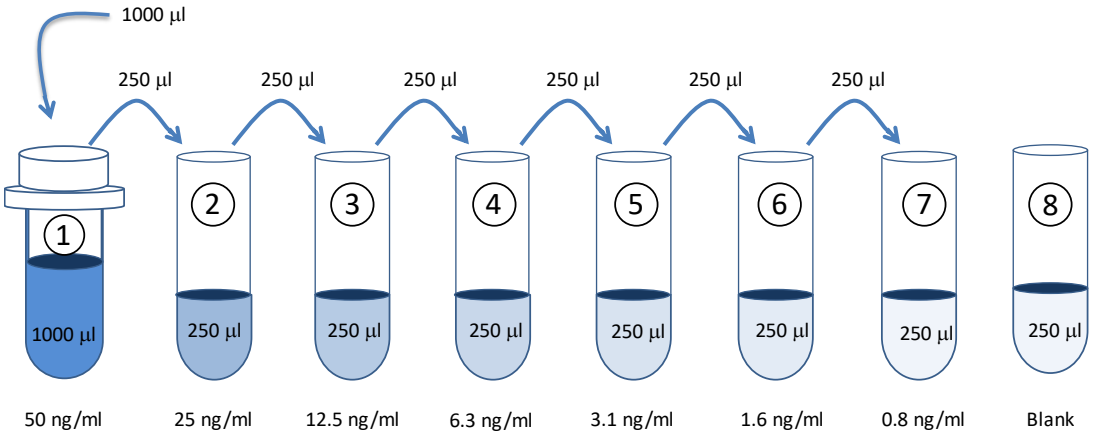
ASSAY PROCEDURE

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**: The recommended standard range is 0.8 - 50 ng/ml. Dissolve one vial of Standard (50 ng/vial) in 1 ml of Sample/Standard/Secondary 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 50 ng/ml solution with an equal volume of Solution B to make a 25 ng/ml solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, and 0.8 ng/ml standard solutions.



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 (1:100 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 1:100 dilution. If serum samples require a lower dilution than 1:100, please contact support@chondrex.com.

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 CPE OR Biotinylated Ara h3**: Prepare biotinylated CPE **OR** Biotinylated Ara h3 with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. *Do not combine biotinylated CPE and biotinylated Ara h3.* Add 100 µl of biotinylated solution to each well and incubate at room temperature for 1.5 hours.

Strip #	Biotinylated Tracer (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

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. 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

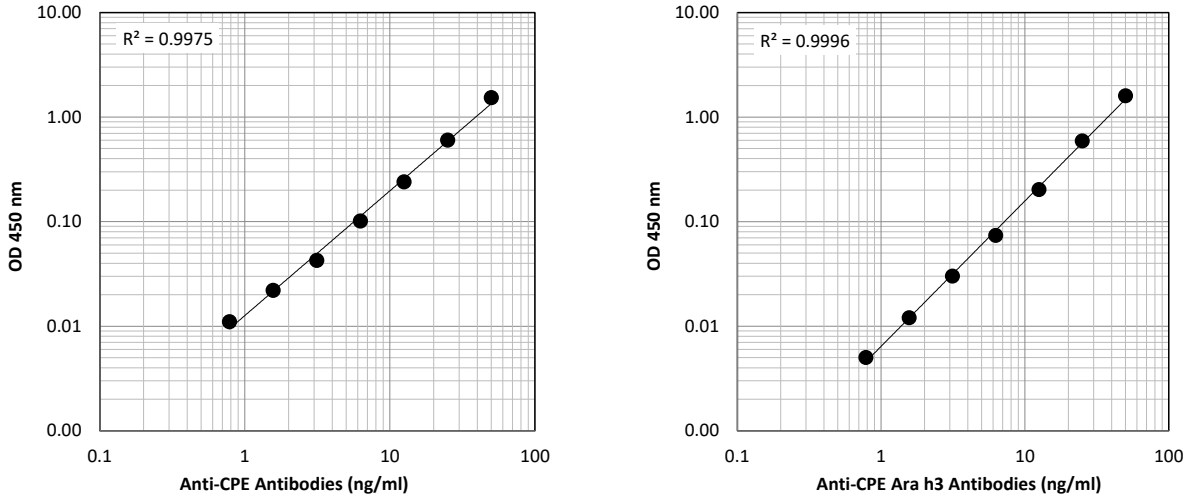
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 and anti-CPE Ara h3 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.

Figure 1 - Typical Standard Curves for the Anti-CPE and Anti-CPE Ara h3 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 h3 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 or anti-CPE Ara h3 IgE antibodies were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay anti-CPE or anti-CPE Ara h3 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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