Mouse Anti- Crude Peanut Extract IgE Antibody ELISA Kit

Catalog # 3063

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-crude peanut extract (CPE) 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:	Intra-Assay (1.1–8.8%)/Inter-Assay (1.2-7.4%)/Spiking Test (92-94%)
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).

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

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse Anti-CPE IgE Antibody (30631)	1 vial	50 ng, lyophilized	-20°C
Biotinylated CPE (30633)	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

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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.

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.

ASSAY OUTLINE

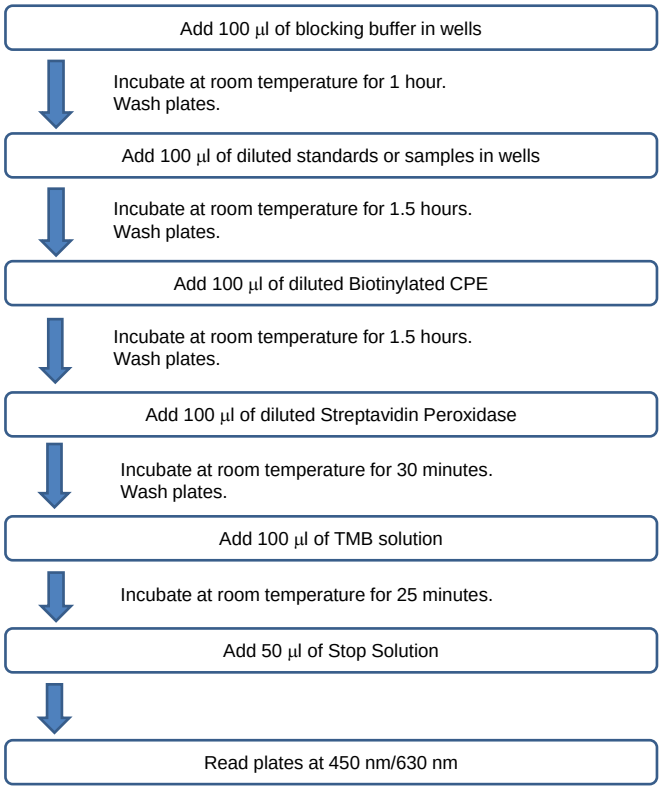

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PLATE MAPPING

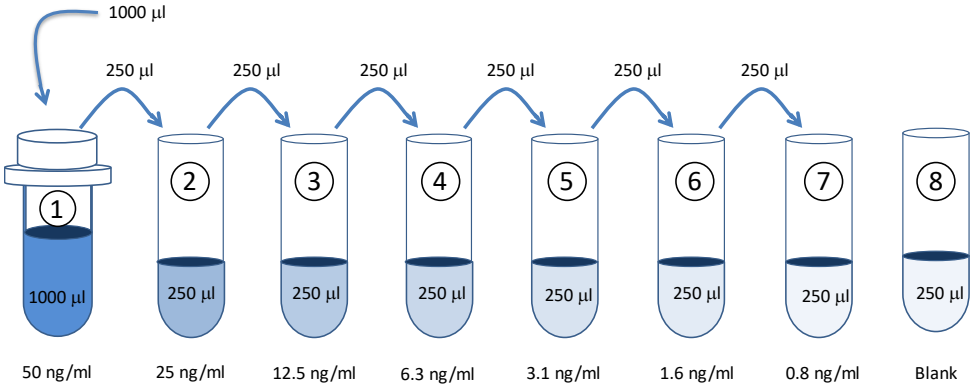
	1	2	3	4	5	6	7	8	9	10	11	12
A	50	50	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	25	25	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	12.5	12.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	6.3	6.3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	3.1	3.1	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	1.6	1.6	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.8	0.8	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards Samples

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**: The dilution of mouse serum immunized with CPE will vary (1:10 or more) depending on the immunization schedule and timing of serum collection. In general, no IgE antibodies against CPE are observed in normal serum at a 1:10 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.

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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**: Prepare biotinylated CPE with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of biotinylated CPE solution to each well and incubate at room temperature for 1.5 hours.

Strip #	Biotinylated CPE (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.

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CALCULATING RESULTS

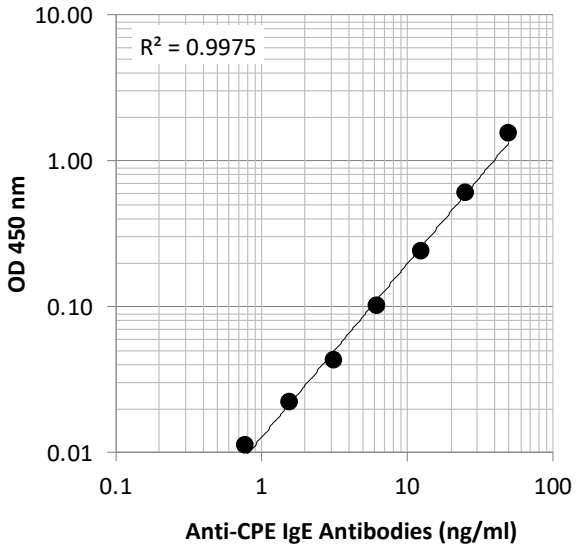
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve for anti-CPE IgE 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 - A Typical Standard Curve for the Anti-CPE IgE Antibody ELISA Kit



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%

*Known amounts of anti-CPE IgE antibodies were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay anti-CPE 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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