Mouse Anti-Crude Peanut Extract Antibody Subtype/Subclass ELISA Kits

Catalog # 3056, 3057, 3058, 3059, 3060, 3061, and 3062

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-crude peanut extract (CPE) antibodies
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	3056 (IgG) : 25 - 0.4 ng/ml 3057 (IgG1) : 50 - 0.8 ng/ml 3058 (IgG2a) : 25 - 0.4 ng/ml 3059 (IgG2b) : 5 - 0.08 ng/ml 3060 (IgG3) : 50 - 0.8 ng/ml 3061 (IgA) : 50 - 0.8 ng/ml 3062 (IgM) : 50 - 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3056: Intra-Assay (3.3-7.4%)/Inter-Assay (4.0-10.4%)/Spiking Test (102-108%) 3057: Intra-Assay (1.9-7.2%)/Inter-Assay (0.8-3.7%)/Spiking Test (93-103%) 3058: Intra-Assay (3.6-4.5%)/Inter-Assay (5.4-9.0%)/Spiking Test (91-110%) 3059: Intra-Assay (4.6-5.8%)/Inter-Assay (4.9-9.8%)/Spiking Test (104-109%) 3060: Intra-Assay (3.1-4.1%)/Inter-Assay (2.3-6.6%)/Spiking Test (104-110%) 3061: Intra-Assay (4.4-9.4%)/Inter-Assay (3.3-5.4%)/Spiking Test (91-105%) 3062: Intra-Assay (3.2-5.8%)/Inter-Assay (3.7-9.4%)/Spiking Test (94-107%)
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 ELISA kits for assaying mouse anti-CPE subtype and subclass antibodies including IgA, IgM, IgG, IgG1, IgG2a, IgG2b, and IgG3 antibodies. Chondrex, Inc. also offers ELISA kits for assaying anti-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	IgG (30561) – 25 ng IgG1 (30571) – 50 ng IgG2a (30581) – 25 ng IgG2b (30591) – 5 ng IgG3 (30601) – 25 ng IgA (30611) – 25 ng IgM (30621) – 50 ng	1 vial	Lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies)	IgG (30113) IgG1 (30133) IgG2a (30153) IgG2b (30163) IgG3 (30393) IgA (30183) IgM (30173)	2 vials	50 µl	-20°C
Solution B - Blocking Buffer (30105)		1 bottle	10 ml	-20°C
Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (30106)		1 bottle	50 ml	-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
Crude Peanut Extract Coated ELISA Plate (Green)		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: Depending on the isotypes, subtypes, and targeting epitopes of antibodies, the binding affinity of individual antibodies varies significantly. Therefore, the total IgG antibody concentration calculated as the sum of individual IgG subtypes might not perfectly match the total IgG concentration as determined by the Mouse Anti-CPE IgG Antibody ELISA Kit (Cat # 3056).

ASSAY OUTLINE

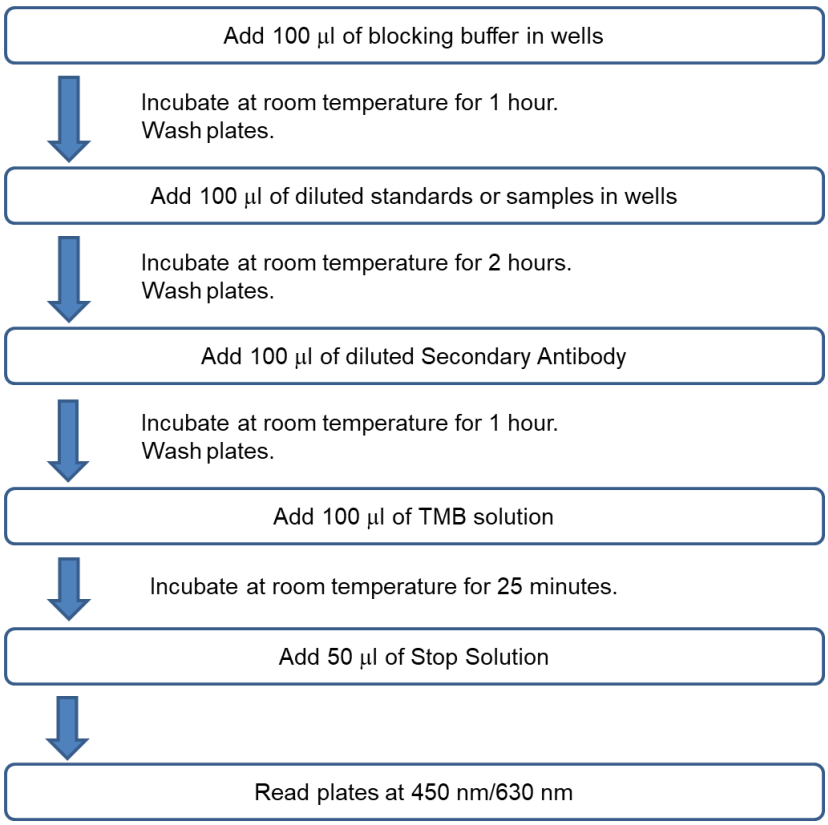

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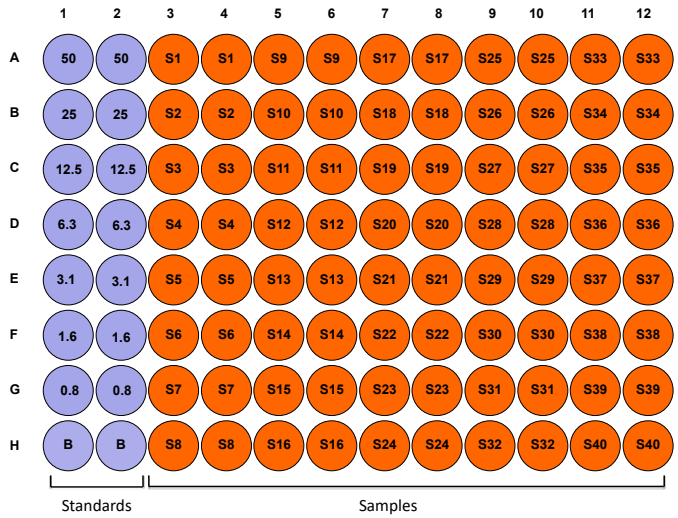
PLATE MAPPING

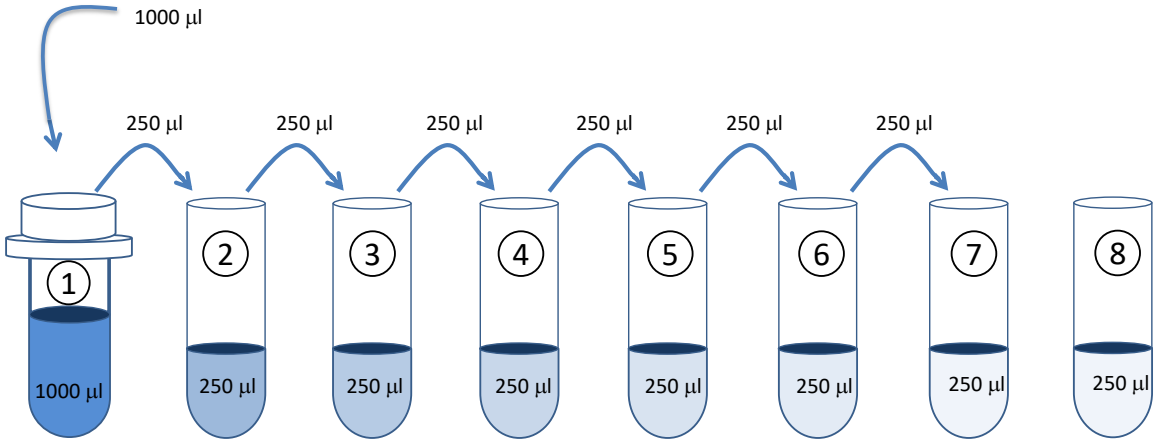
Example of the Mouse Anti-CPE IgG1, IgG3, IgA, and IgM Antibody ELISA Kits



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/Secondary Antibody Dilution Buffer (Solution C) and keep it as a standard stock. Dilute the standard stock accordingly to get the first stock solution. Then serially dilute it with Solution C. For example, mix 250 µl of the first stock solution with an equal volume of Solution C to make the second stock solution, and then repeat it five more times. The original standard stock solution may be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



IgG2b:	5 ng/ml	2.5 ng/ml	1.3 ng/ml	0.63 ng/ml	0.31 ng/ml	0.16 ng/ml	0.08 ng/ml	Blank
IgG/ IgG2a:	25 ng/ml	12.5 ng/ml	6.3 ng/ml	3.1 ng/ml	1.6 ng/ml	0.8 ng/ml	0.4 ng/ml	Blank
IgG1/IgG3/IgA/IgM:	50 ng/ml	25 ng/ml	12.5 ng/ml	6.3 ng/ml	3.1 ng/ml	1.6 ng/ml	0.8 ng/ml	Blank

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3. **Prepare Sample Dilutions**: The dilution of serum from mice immunized with CPE 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.

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 C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 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 Secondary Antibody**: Dilute one vial of Secondary Antibody in 10 ml Sample/Standard/Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	2nd Antibody (µl)	Solution C (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

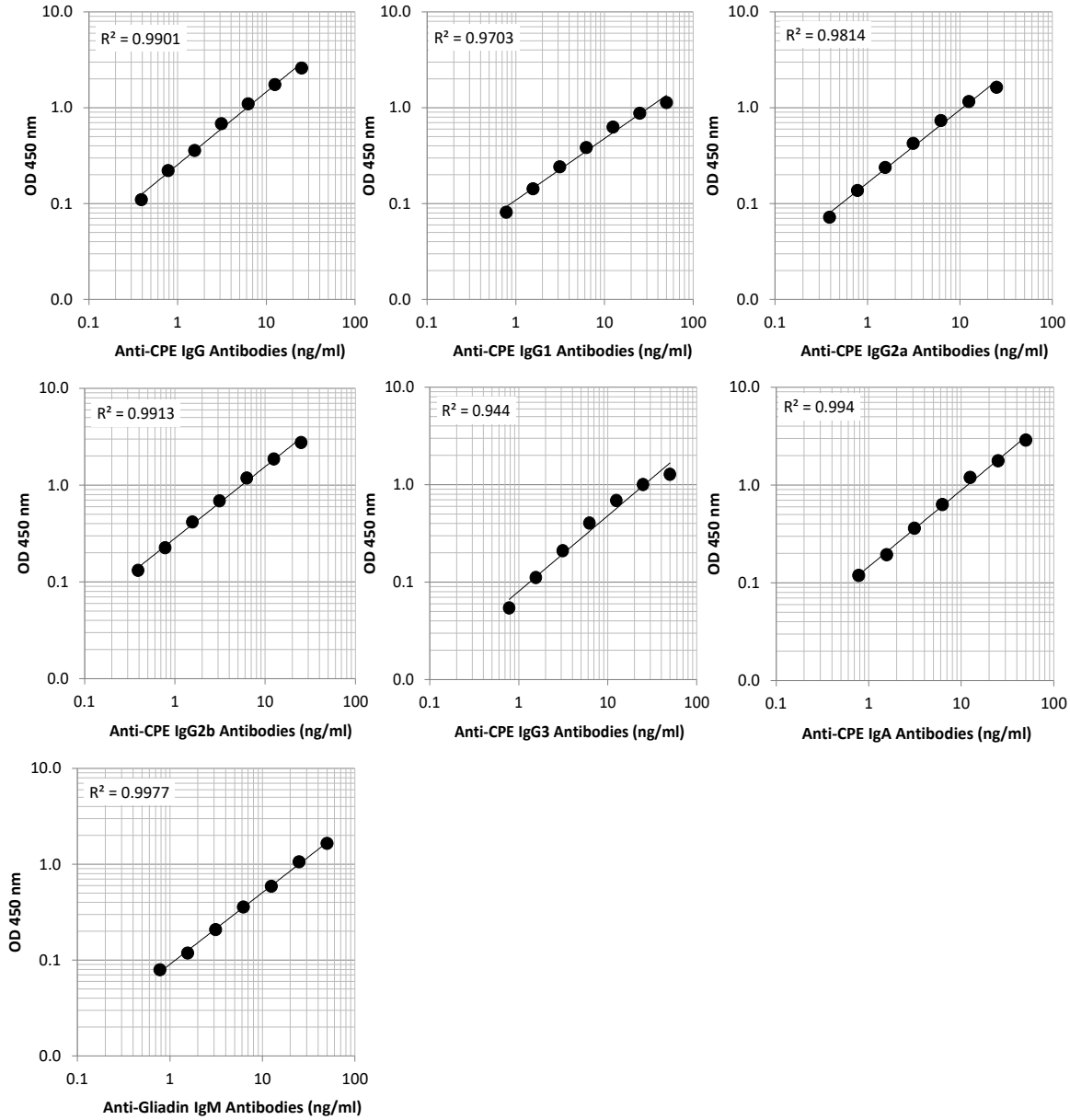
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows sample standard curves for anti-CPE Ig 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 original test samples.

Figure 1 - Typical Standard Curves for the Anti-CPE Antibody ELISA Kits



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VALIDATION DATA

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

Test	0.5 ng/ml	2.5 ng/ml	10 ng/ml
Intra-Assay CV (%)	4.3	7.4	3.3
Inter-Assay CV (%)	9.3	4.0	10.4
Spike Test* (%)	102%	108%	107%

Table 2 - Reproducibility Data for the Mouse Anti-CPE IgG1 Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	7.1	7.2	1.9
Inter-Assay CV (%)	3.7	3.3	0.8
Spike Test* (%)	95%	93%	103%

Table 3 - Reproducibility Data for the Mouse Anti-CPE IgG2a Antibody ELISA Kit

Test	1.0 ng/ml	6.0 ng/ml	20 ng/ml
Intra-Assay CV (%)	3.6	4.2	4.5
Inter-Assay CV (%)	9.0	5.4	7.9
Spike Test* (%)	95%	91%	110%

Table 4 - Reproducibility Data for the Mouse Anti-CPE IgG2b Antibody ELISA Kit

Test	0.1 ng/ml	0.63 ng/ml	2.5 ng/ml
Intra-Assay CV (%)	5.8	4.6	5.6
Inter-Assay CV (%)	9.8	5.7	4.9
Spike Test* (%)	106%	104%	109%

Table 5 - Reproducibility Data for the Mouse Anti-CPE IgG3 Antibody ELISA Kit

Test	1.5 ng/ml	8 ng/ml	35 ng/ml
Intra-Assay CV (%)	3.3	4.1	3.1
Inter-Assay CV (%)	6.6	2.3	4.5
Spike Test* (%)	110%	105%	104%

Table 6 - Reproducibility Data for the Mouse Anti-CPE IgA Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	7.1	9.4	4.4
Inter-Assay CV (%)	3.4	5.4	3.3
Spike Test* (%)	105%	96%	91%

Table 7 - Reproducibility Data for the Mouse Anti-CPE IgM Antibody ELISA Kit

Test	1.3 ng/ml	8 ng/ml	20 ng/ml
Intra-Assay CV (%)	3.2	5.4	5.8
Inter-Assay CV (%)	4.8	3.7	9.4
Spike Test* (%)	107%	101%	94%

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

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TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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