Mouse Anti-Peanut Ara h 1 Antibody Subtype/Subclass ELISA Kits

Catalog # 3084, 3085, 3086, 3087, and 3088

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-peanut Ara h 1 antibodies
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	3084 (IgG) : 25 - 0.4 ng/ml
	3085 (IgG1) : 50 - 0.8 ng/ml
	3088 (IgG2a) : 50 - 0.8 ng/ml
	3086 (IgG2b) : 12.5 - 0.2 ng/ml
	3087 (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:	3084: Intra-Assay (1.7-5.0%)/Inter-Assay (3.9-7.7%)/Spiking Test (87-97%)
	3085: Intra-Assay (0.9-3.6%)/Inter-Assay (3.6-9.2%)/Spiking Test (96-107%)
	3088: Intra-Assay (2.4-5.7%)/Inter-Assay (5.0-8.4%)/Spiking Test (101-104%)
	3086: Intra-Assay (4.4-5.3%)/Inter-Assay (6.3-7.9%)/Spiking Test (89-94%)
	3087: Intra-Assay (2.8-6.0%)/Inter-Assay (4.6-7.3%)/Spiking Test (92-96%)
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 h6 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*).

To evaluate the humoral immune response against Ara h 1 in mouse allergy models, Chondrex, Inc. provides ELISA kits for assaying mouse anti-Ara h 1 subtype and subclass antibodies including IgG, IgG1, and IgG2b antibodies. 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

Antibody ELISA Kit	CPE Catalog #	Ara h 1 Catalog #	Ara h 2 Catalog #	Ara h 3 Catalog #
Mouse Anti-Peanut IgG	3056	3084	3077	3082
Mouse Anti-Peanut IgG1	3057	3085	Coming Soon!	3083
Mouse Anti-Peanut IgG2b	3059	3086	3078	Coming soon!
Mouse Anti-Peanut IgG2a	3058	3088	Coming soon!	Coming soon!
Mouse Anti-Peanut IgG3	3060	Coming soon!	Coming soon!	Coming soon!
Mouse Anti-Peanut IgA	3061	Coming soon!	3080	Coming soon!
Mouse Anti-Peanut IgM	3062	3087	3079	Coming soon!
Mouse Anti-Peanut IgE	3063	Coming soon!	Coming soon!	3071

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KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard	IgG (30841) – 25 ng IgG1 (30851) – 50 ng IgG2a (30881) – 50 ng IgG2b (30861) – 12.5 ng IgM (30871) – 50 ng	1 vial	Lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies)	IgG (30113) IgG1(30133) IgG2a (30153) IgG2b (30163) 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
Recombinant Ara h 1 Coated ELISA Plate (Pink)		1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE

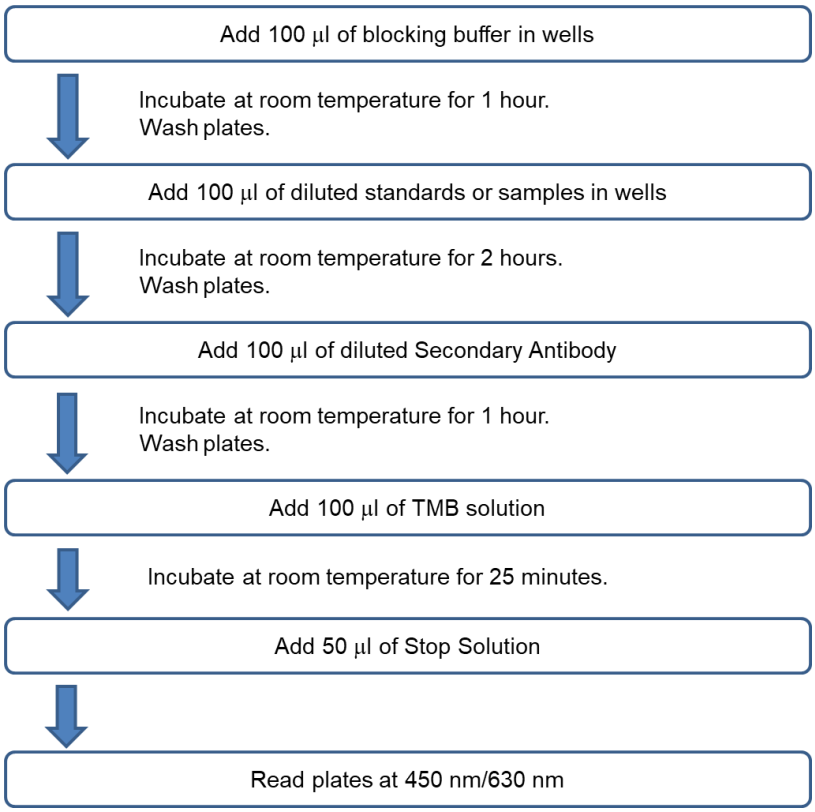

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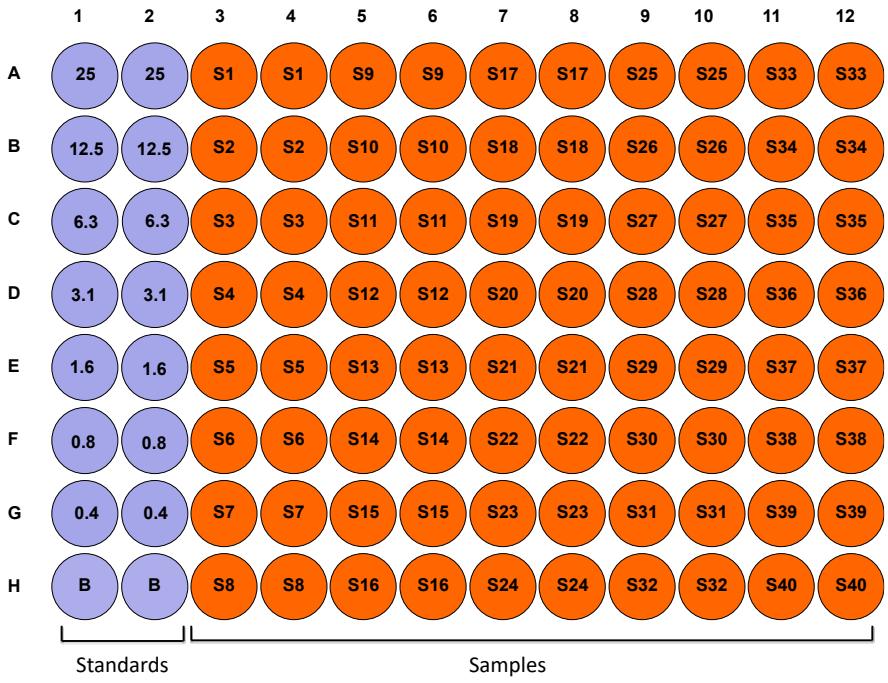
PLATE MAPPING

Example of the Mouse Anti-Ara h 1 IgG Antibody ELISA Kits



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.

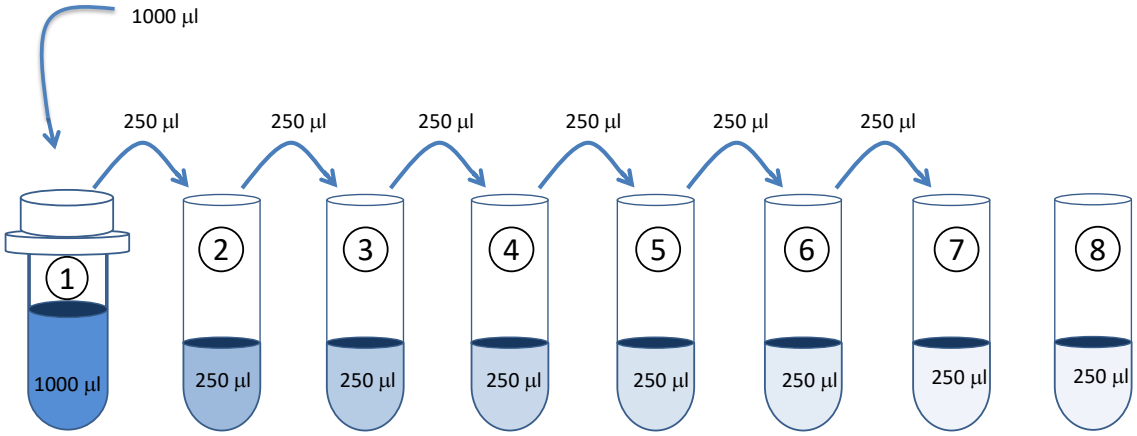
NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

NOTE 8: Depending on the isotypes, subtypes, and targeting epitopes of antibodies, the binding affinity of individual antibodies varies significantly. For example, the total CPE IgG antibody concentration calculated as the sum of individual anti-CPE IgG subtypes might not perfectly match the total IgG concentration as determined by the Mouse Anti-Ara h 1 IgG Antibody ELISA Kit (Cat # 3084).

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 -20°C for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



IgG:	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/IgG2a/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
IgG2b:	12.5 ng/ml	6.3 ng/ml	3.1 ng/ml	1.6 ng/ml	0.8 ng/ml	0.4 ng/ml	0.2 ng/ml	Blank

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 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.

CALCULATING RESULTS

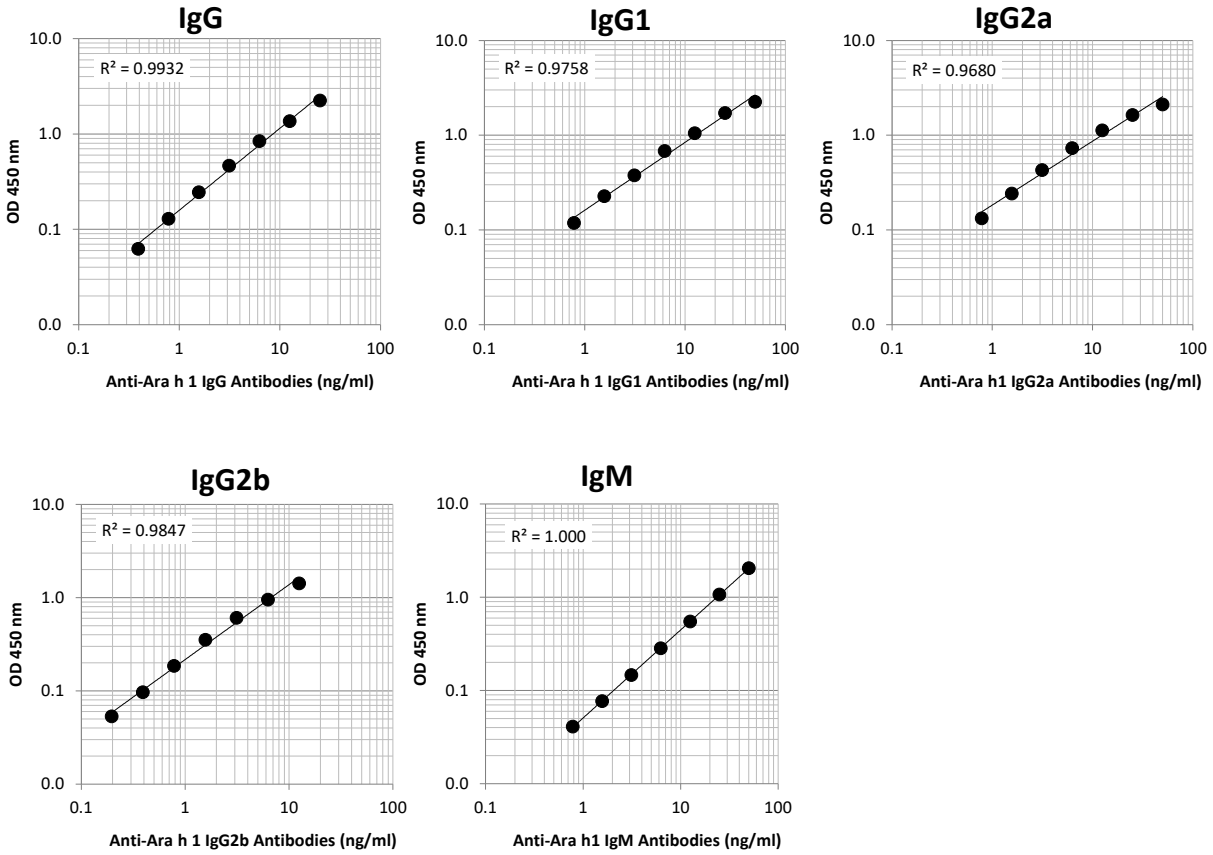
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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-Ara h 1 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-Ara h 1 Antibody ELISA Kits



VALIDATION DATA

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

Test	0.8 ng/ml	3.1 ng/ml	12.5 ng/ml
Intra-Assay CV (%)	5.0	1.7	2.0
Inter-Assay CV (%)	7.7	3.9	5.4
Spike Test* (%)	87%	97%	97%

Table 2 - Reproducibility Data for the Mouse Anti-Ara h 1 IgG1 Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	3.6	2.1	0.9
Inter-Assay CV (%)	9.2	3.7	3.6
Spike Test* (%)	99%	96%	107%

Table 3 - Reproducibility Data for the Mouse Anti-Ara h 1 IgG2a Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	5.7	2.4	5.7
Inter-Assay CV (%)	8.4	5.4	5.0
Spike Test* (%)	102%	101%	104%

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Table 4 - Reproducibility Data for the Mouse Anti-Ara h 1 IgG2b Antibody ELISA Kit

Test	0.4 ng/ml	1.6 ng/ml	6.3 ng/ml
Intra-Assay CV (%)	4.4	4.6	5.3
Inter-Assay CV (%)	6.3	7.9	6.4
Spike Test* (%)	94%	89%	90%

Table 5 - Reproducibility Data for the Mouse Anti-Ara h 1 IgM Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	6.0	2.8	3.7
Inter-Assay CV (%)	7.3	4.6	5.4
Spike Test* (%)	92%	94%	96%

TROUBLESHOOTING

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

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