References

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2. Maloney JM, Chapman MD, Sicherer SH. Peanut allergen exposure through saliva: assessment and interventions to reduce exposure. J Allergy Clin Immunol. 2006 Sep;118(3):719-24.
3. Zhuang Y, Dreskin SC. Redefining the major peanut allergens. Immunol Res. 2013 March; 55(0): 125-34.
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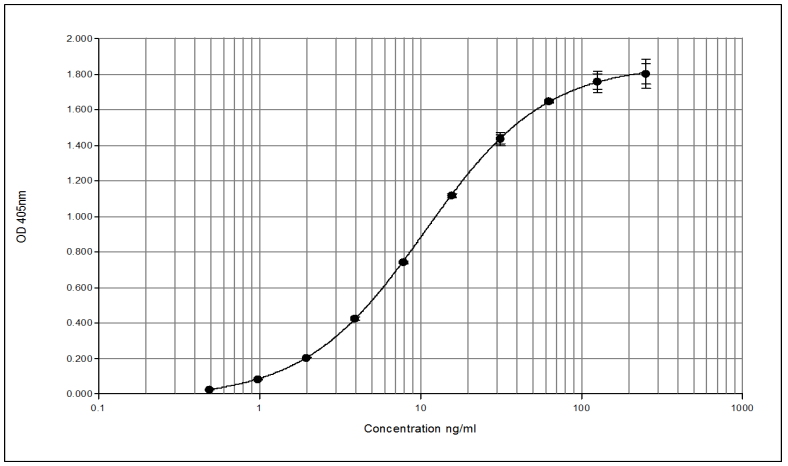
INDOOR biotechnologies INC

Ara h 8 ELISA kit (4G6/AH8)

Product Code: EL-AH8

Lot Number: XXXXX

Sample Curve:



Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 4G6
Concentration: 1.0 mg/ml

Vial 2 (white top) 400 µL
Ara h 8 Standard
Concentration: 2,500 ng/ml

Vial 3 (brown) 100 µL
Polyclonal Ara h 8 rabbit antiserum
Dilute: 1/1,000 for use

Storage: The ELISA kit should be stored at 4°C

For research and commercial use in vitro: not for human in vivo or therapeutic use.

An InBio™ product

Certificate of Analysis

Capture antibody:	4G6
Immunogen:	Ara h 8
Isotype:	Mouse IgG
Specificity:	Binds to an epitope present on *Arachis hypogaea* allergen, Ara h 8. Cross-reactive with structurally-homologous birch pollen allergen, Bet v 1.
Purification:	Produced *in vitro* and purified by chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.
Concentration:	1.0 mg/ml in phosphate buffered saline, pH 7.4. Based on A280 for IgG (1.42=1mg/ml) 0.22µm filtered, preservative free.
Lot Number:	**XXXXX**

Detection antibody:	Rabbit polyclonal antiserum
Immunogen:	Ara h 8
Isotype:	Multiple
Specificity:	The antiserum contains IgG antibodies to Ara h 8. Minimal cross-reactivity with structurally-homologous birch pollen allergen, Bet v 1.
Activity:	The antiserum has been titrated for use in ELISA at 1/1,000 dilution. Prepared in 1% BSA/50% glycerol/ PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number:	**XXXXX**

Allergen Standard:	Recombinant Ara h 8 prepared in 1% BSA/50% glycerol/PBS, pH 7.4
Concentration:	2,500 ng/ml based on amino acid analysis.
Lot Number	**XXXXX**

ELISA Protocol for Ara h 8

1. Coat polystyrene microtiter plate (NUNC Maxisorp catalog # 439454) with 100µl mAb 4G6 stock at 1/1,000 dilution in 50mM carbonate-bicarbonate buffer, pH 9.6 (i.e. 10µl/10ml). Incubate overnight at 4°C.

2. Wash the plate 3x with 150µl/well PBS-0.05% Tween 20, pH 7.4 (PBS-T). Tap the plate on absorbent paper to remove residual buffer. Add 100µl/well PBS-T,1% BSA, and incubate for 30 min. at room temperature.

3. Empty the plate and tap on absorbent paper to remove residual buffer. Prepare a control curve by making two-fold dilutions of the Ara h 8 standard ranging from 250 - 0.49ng/ml: pipette 20µl Ara h 8 standard into 180µl PBS-T, 1% BSA, into wells A1 and B1 on the ELISA plate. Mix well and transfer 100µl into 100µl PBS-T, 1% BSA in wells A2 and B2. Mix well and continue making doubling dilutions across the plate through wells A10 and B10. Wells A11, B11 and A12, B12 should contain only 100µl PBS-T, 1% BSA as blanks.

4. Add 100µl of diluted allergen samples to the plate: house dust extracts are routinely diluted two-fold starting at 1/10. Other sample types, like food extracts and allergen extracts, may require different dilutions. It is recommended to test at least three dilutions of each sample. Incubate for 1 hour at room temperature.

5. Wash the plate 3x with 150µl/well PBS-T. Dilute the polyclonal Ara h 8 antiserum 1/1,000 in PBS-T, 1% BSA and add 100µl to each well. Incubate for 1 hour at room temperature.

6. Wash the plate 3x with 150µl/well PBS-T. Dilute peroxidase-conjugated goat anti-rabbit IgG (Jackson Laboratories Cat# 111-036-046, reconstituted in 1 ml distilled water and 1ml glycerol) 1/1,000 in PBS-T, 1% BSA and incubate for 1 hour at room temperature.

7. Wash the plate 3x with 150µl/well PBS-T and develop the assay by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1,000 dilution of H_2O_2*. Read the plate when the absorbance at 405nm reaches 2.0-2.4.
   *H_2O_2 should be added to ABTS immediately prior to plate development

Notes:

The Ara h 8 Standard is recommended for immunoassay calibration purposes only. Not recommended for in-vitro antibody measurements, T cell studies, immunization purposes, or other uses.

Buffer recipes, storage conditions and a list of frequently asked questions can be found under "Protocols" on our web site: www.inbio.com.

<u>For research and commercial use in vitro: not for human in vivo or therapeutic use.</u>