

## References

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3. Perry TT, Conover-Walker MK, Pomés A, Chapman MD, Wood RA. Distribution of peanut allergen in the environment. *J Allergy Clin Immunol.* 2004 May;113(5):973-6.
4. 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.



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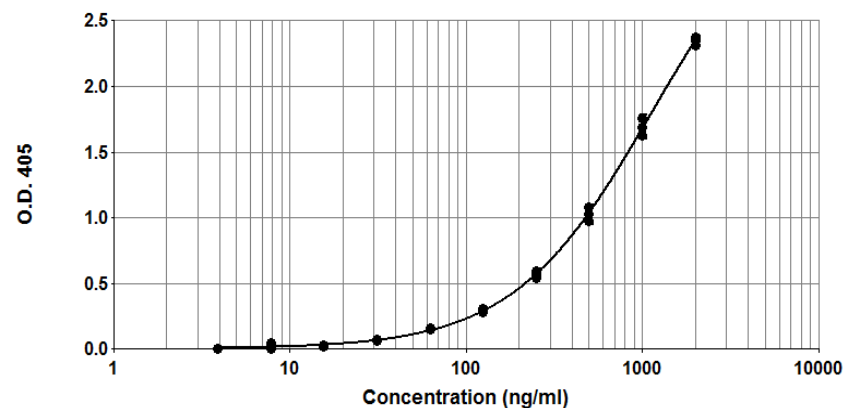


## Ara h 1 ELISA kit (2C12/2F7)

Product Code: EL-AH1

Lot Number: xxxxx

### Sample Curve:



### Content:

- Vial 1 (red top) 100  $\mu$ L  
Monoclonal antibody 2C12  
Concentration: 2.7mg/ml in PBS
- Vial 2 (white top) 400  $\mu$ L  
Ara h 1 Standard  
Concentration: 20,000ng/ml nAra h 1
- Vial 3 (brown) 100  $\mu$ L  
Biotinylated monoclonal antibody 2F7  
Dilute: 1:1000 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.**

## Certificate of Analysis

Monoclonal Antibody: 2C12 (clone 2C12 A11 A3)  
Immunogen: Ara h 1  
Isotype: Mouse IgG<sub>1</sub>  
Specificity: Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 1.  
Purification: Produced *in vitro* by BioVectra dcl bioreactors and purified by chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.  
Concentration: 2.7 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**

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Monoclonal Antibody: 2F7 (clone 2F7 C12 D10)  
Immunogen: Ara h 1  
Isotype: Mouse IgG<sub>1</sub>  
Specificity: Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 1.  
Purification: Produced in ascites and purified by affinity chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.  
Biotinylation: Biotinylated and titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.  
**Lot Number: xxxxx**

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Allergen Standard: nAra h 1  
Composition: Naturally purified Ara h 1 prepared in 1% BSA/50% glycerol/PBS, pH 7.4  
Concentration: 20,000ng/ml  
Calibration: The Ara h 1 concentration of the purified Ara h 1 was determined by OD<sub>280</sub>.  
Stability/Storage: **Please store the standard at -20°C (±5°C)**  
**Lot Number xxxxx**

## ELISA Protocol for Ara h 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 2C12 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.
2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100µl/well of 1% BSA, PBS-T. Wash 3x with PBS-T.
3. Use doubling dilutions of the nAra h 1 standard to make a control curve ranging from 2000 - 4ng/ml Ara h 1: Pipette 20µl Ara h 1 standard into 180µl 1% BSA, PBS-T into wells A1 and B1 on the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA, PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11 and A12, B12 should contain only 1% BSA, PBS-T as blanks.
4. Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Ara h 1 analysis are routinely diluted two-fold from 1/10-1/80. Other sample types, like air filter extracts and allergen extracts, may require different dilutions.
5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Ara h 1 mAb 2F7. The antibody solution contains 50% glycerol and should be diluted 1/1000 in 1%BSA, PBS-T. Incubate for 1 hour at room temperature.
6. Wash wells 3x with PBS-T and add 100µl diluted Streptavidin-Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The reconstituted Streptavidin should be diluted 1/1000 in 1% BSA, PBS-T. Incubate for 30 minutes at room temperature.
7. Wash wells 3x and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H<sub>2</sub>O<sub>2</sub>. Read the plate when the absorbance at 405nm reaches 2.0-2.4.

### Notes:

*The Ara h 1 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](http://www.inbio.com).*

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