

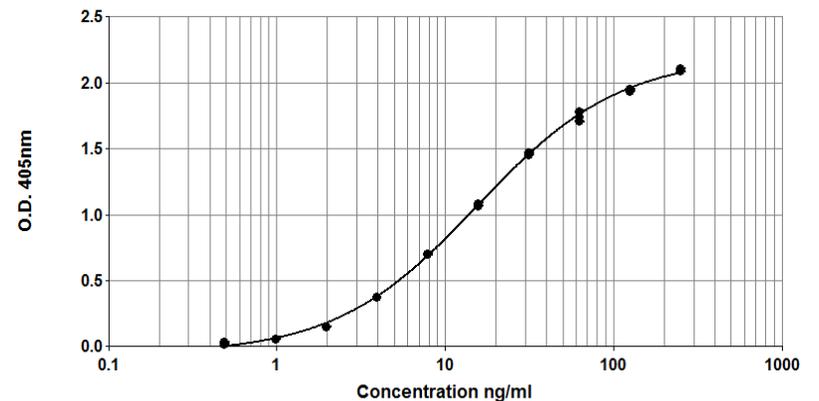


## Ara h 2 ELISA kit (1C4/AH2)

Product Code: EL-AH2

Lot Number: xxxxx

### Sample Curve:



### Content:

- Vial 1 (red top) 100  $\mu$ L  
Monoclonal antibody 1C4  
Concentration: 2mg/ml in PBS
- Vial 2 (white top) 400  $\mu$ L  
Ara h 2 Standard  
Concentration: 2500ng/ml Ara h 2
- Vial 3 (brown) 100  $\mu$ L  
Rabbit anti Ara h 2 antibody  
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.**



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## Certificate of Analysis

Monoclonal Antibody: 1C4 (clone 1C4 G4 C8)  
Immunogen: Ara h 2  
Isotype: Mouse IgG1  
Specificity: Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 2.  
Purification: Produced in ascites and purified by affinity chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.  
Concentration: 2 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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Antibody: Polyclonal rabbit antiserum raised against natural purified Ara h 2  
Specificity: The antiserum contains IgG antibodies to Ara h 2  
Activity: The antiserum has been diluted in phosphate buffered saline, pH 7.4, containing 1%BSA/50% glycerol. The antiserum has been 0.22µm filtered and should be diluted 1/1000 for Ara h 2 ELISA.  
Lot Number: **xxxxx**

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Allergen Standard: nAra h 2  
Composition: Naturally purified Ara h 2 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.  
Concentration: 2500ng/ml  
Calibration: The Ara h 2 concentration of the purified Ara h 2 was determined by O.D. <sub>280</sub>.  
Lot Number **xxxxx**

## ELISA Protocol for Ara h 2.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 1C4 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 2 standard to make a control curve ranging from 250 - 0.5ng/ml Ara h 2: Pipette 20µl Ara h 2 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 2 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 polyclonal Rabbit anti Ara h 2 antibody. 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 Peroxidase conjugated Goat anti-Rabbit IgG (Jackson Laboratories Cat# 111-036-046, reconstituted in 1 ml distilled water and 1ml glycerol). The reconstituted Goat anti-Rabbit IgG should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA, PBS-T. Incubate for 1hour 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 2 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).*

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