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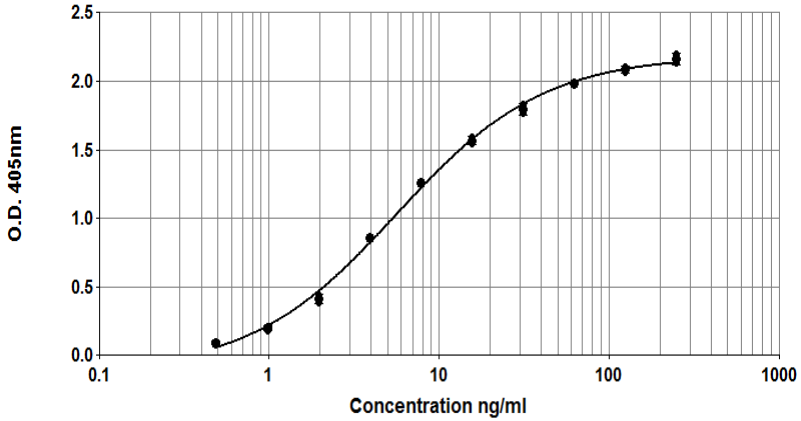
Ara h 2 ELISA kit (1C4/AH2)

Product Code: EL-AH2

Lot Number: XXXXX

Sample Curve:



Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 1C4
Concentration: 2.0mg/ml in PBS

Vial 2 (white top) 400 µL
Ara h 2 Standard
Concentration: 2500ng/ml Ara h 2

Vial 3 (brown) 100 µL
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.

An InBio™ product

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:	2.0 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**

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**

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 amino acid analysis.
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 from1/10-1/80. 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_2O_2. 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.

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

For Research Use Only: Not for Diagnostic or Therapeutic Use