References

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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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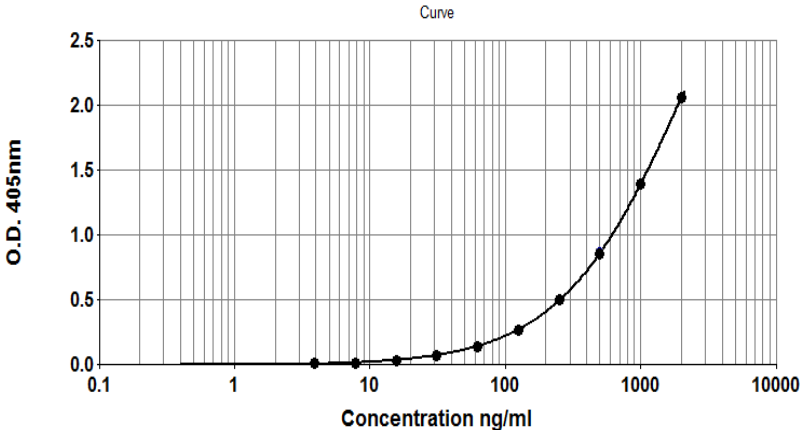
INDOOR biotechnologies INC

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

Product Code: EL-AH1

Lot Number: XXXXX

Sample Curve:



Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 2C12

Vial 2 (white top) 400 µL
Ara h 1 Standard
Concentration: 20,000 ng/ml nAra h 1

Vial 3 (brown) 100 µL
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.

An InBio™ product

Certificate of Analysis

Monoclonal Antibody:	2C12 (clone 2C12 A11 A3)
Immunogen:	Ara h 1
Isotype:	Mouse IgG_1
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:	1.9 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**

Monoclonal Antibody:	2F7 (clone 2F7 C12 D10)
Immunogen:	Ara h 1
Isotype:	Mouse IgG_1
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**

Allergen Standard:	nAra h 1
Composition:	Naturally purified Ara h 1 prepared in 1% BSA/30% 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_{280}.
Stability/Storage:	**Store standard at –20°C (±5°C)** ****Expiry 6 months from receipt****
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 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 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_2O_2. 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.

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