References

1. Pomés A, Helm RM, Bannon GA, Burks AW, Tsay A, Chapman MD. Monitoring peanut allergen in food products by measuring Ara h 1. J Allergy Clin Immunol 2003;111:640-5.

2. Pomés A, Vinton R, Chapman MD. Peanut allergen (Ara h 1) detection in foods containing chocolate. J Food Prot. 2004 Apr; 67 (4):793-8.

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.

INDOOR biotechnologies INC

www.inbio.com

Indoor Biotechnologies, Inc.
700 Harris Street,
Charlottesville, VA 22903
United States

Tel: (434) 984-2304
Fax:(434) 984-2709
Email: mail@inbio.com

Indoor Biotechnologies Ltd.
Vision Court
Caxton Place
Cardiff, Wales CF23 8HA
United Kingdom

Tel: +44 (0) 29 2167 4640
Email: Info@indoorbiotech.co.uk

Indoor Biotechnologies,
India Private Limited
Bangalore Bioinnovation Centre,
BioTech Park, Electronic City Phase 1
Bangalore-560100, India

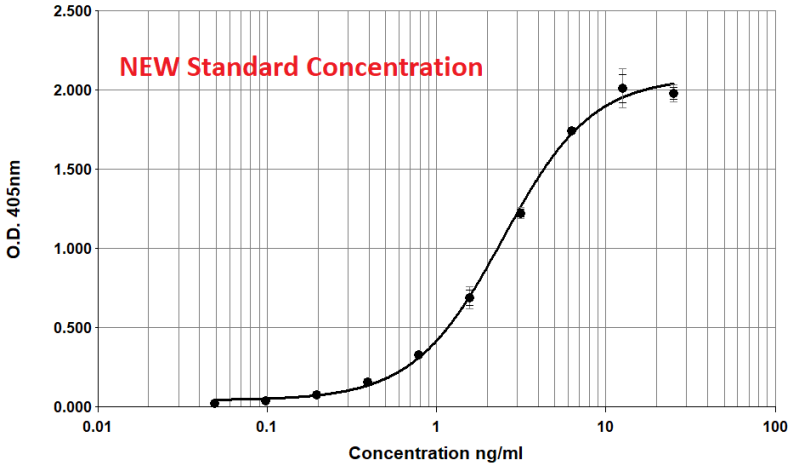
Tel: +91-9901722009
Email: info@inbioindia.com

INDOOR biotechnologies INC

Ara h 6 ELISA kit (3B8/3E12)

Product Code: EL-AH6

Lot Number: XXXXX



Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 3B8

Vial 2 (white top) 400 µL
nAra h 6 Standard
Concentration: **250ng/ml**

Vial 3 (brown) 100 µL
Biotinylated monoclonal antibody 3E12
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:	3B8 (clone 3B8 B5)
Immunogen:	Ara h 6
Isotype:	Mouse IgG1
Specificity:	Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 6.
Purification:	Produced in ascites and purified by chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.
Concentration:	1.5mg/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:	3E12 (clone 3E12 C4 B3)
Immunogen:	Ara h 6
Isotype:	Mouse IgG1
Specificity:	Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 6.
Purification:	Produced in ascites and purified by 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 6
Composition:	Naturally purified Ara h 6 prepared in 1% BSA, 50% glycerol/PBS, pH 7.4
Concentration:	**250 ng/ml**
Calibration:	The concentration of the purified natural Ara h 6 was determined by amino acid analysis.
Lot Number	**xxxxx**

ELISA Protocol for Ara h 6

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 3B8 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 6 standard to make a control curve ranging from 25 - 0.05ng/ml Ara h 6: Pipette 20µl Ara h 6 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 6 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 6 mAb 3E12. 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 and add 100µl diluted Streptavidin - Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The Streptavidin Peroxidase 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 6 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.