

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

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

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

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

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

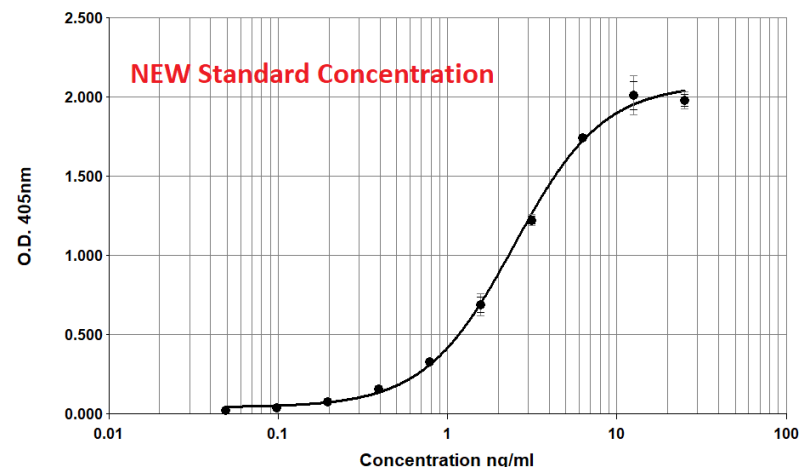
Tel: +91-9901722009
Email: info@inbioindia.com



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

Product Code: EL-AH6

Lot Number: xxxxx



Content:

Vial 1 (red top) 100 μ L
Monoclonal antibody 3B8
Concentration: 2mg/ml in PBS

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

Vial 3 (brown) 100 μ L
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 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 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 H₂O₂. 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.

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