

References

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3. Namork E, Stensby BA. Peanut sensitization pattern in Norwegian children and adults with specific IgE to peanut show age related differences. *Allergy Asthma Clin Immunol*. 2015 Nov;11:32.



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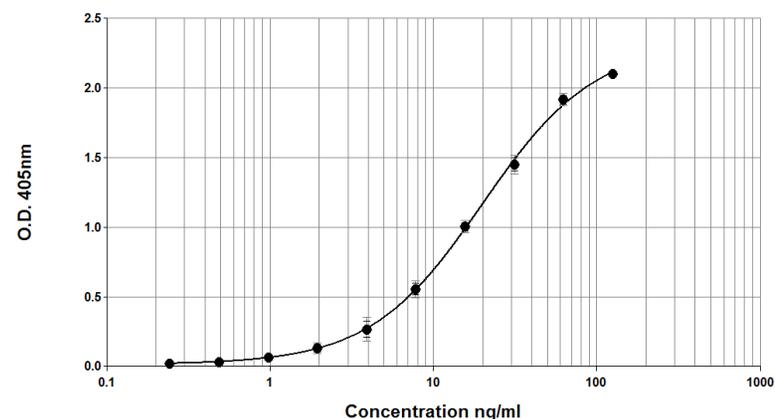


Ara h 3 ELISA kit (1E8/4G9)

Product Code: EL-AH3

Lot Number: XXXXX

Sample Curve:



Content:

- Vial 1 (red top) 100 μ L
Monoclonal antibody 1E8
Concentration: 1.1 mg/ml in PBS
- Vial 2 (white top) 400 μ L
Ara h 3 Standard
Concentration: 1,250 ng/ml nAra h 3
- Vial 3 (brown) 100 μ L
Biotinylated monoclonal antibody 4G9
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

ELISA Protocol for Ara h 3.

Certificate of Analysis

Monoclonal Antibody: 1E8
Immunogen: Ara h 3
Isotype: Mouse IgG₁
Specificity: Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 3.
Purification: Produced *in vitro* and purified by affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration: 1.1 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: 4G9
Immunogen: Ara h 3
Isotype: Mouse IgG₁
Specificity: Binds to species specific epitope present on *Arachis hypogaea* allergen, Ara h 3.
Purification: Produced *in vitro* and purified by affinity chromatography using Protein A. 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 3
Composition: Purified natural Ara h 3 prepared in 1% BSA/50% glycerol/PBS, pH 7.4
Concentration: 1,250ng/ml
Calibration: The Ara h 3 concentration of the purified nAra h 3 was determined by amino acid analysis.
Storage: **Store the standard at -20°C (±5°C)**
Lot Number XXXXX

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 1E8 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 3 standard to make a control curve ranging from 125– 0.24ng/ml Ara h 3: Pipette 20µl Ara h 3 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 3 analysis are routinely diluted two-fold from 1/10-1/80. Other sample types, like air filter extracts, food product extracts and allergen extracts, may require different dilutions.
5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Ara h 3 mAb 4G9. 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 H₂O₂. Read the plate when the absorbance at 405nm reaches 2.0-2.4.

Notes:

The Ara h 3 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.