Assay Performance Characteristics:

Standard range: 100.0-0.2ng/mL
Limit of Detection: 0.78ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

Plate Template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

References:

www.inbio.com

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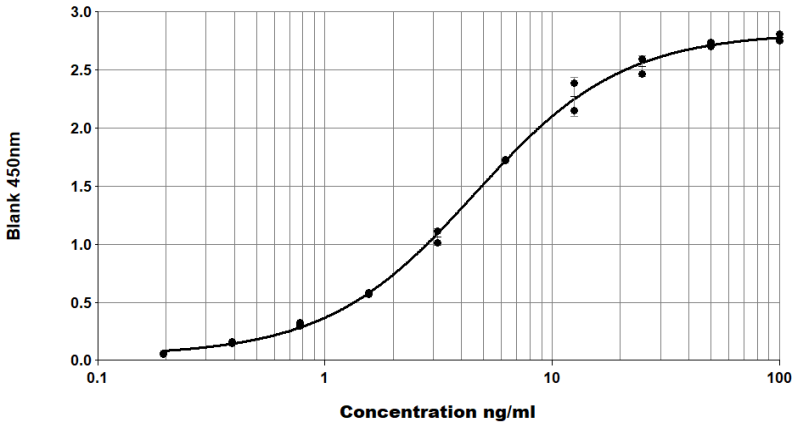
Ar a h 8 ELISA 2.0

Pre-coated Plate Kit

Product Code: EPC-AH8-X
Lot Number: xxxxx

Sample curve:



Contents:
Microtiter plate coated with anti-Ara h 8 monoclonal antibody 4G6

Ara h 8 allergen standard (white cap)
Concentration: 1000ng/mL

Rabbit anti Ara h 8 polyclonal antiserum (brown cap)

Peroxidase-conjugated Goat Anti-Rabbit IgG (blue cap)

Wash buffer (10x concentrate)
Assay buffer (10x concentrate)
TMB developing substrate
Stop solution (0.5N sulfuric acid)

Store kit at 2-8^{O}C
Expiry:

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

An InBio™ product

***Additional step required, see protocol steps #6-9**

Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody 4G6 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
Monoclonal Antibody:	4G6
Immunogen:	Ara h 8
Isotype:	Mouse IgG1
Specificity:	Binds to an epitope present on *Arachis hypogaea* allergen, Ara h 8. Cross-reactive with structurally-homologous birch pollen allergen, Bet v 1.
Purification:	Produced in cell culture and purified by affinity chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.
Lot Number:	xxxxx
Detection Antibody:	Rabbit polyclonal antiserum
Immunogen:	Ara h 8
Isotype:	Multiple
Specificity:	The antiserum contains IgG antibodies to Ara h 8. Minimal cross-reactivity with structurally-homologous birch pollen allergen, Bet v 1.
Activity:	The antiserum has been titrated for use in ELISA at 1/1,000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number:	xxxxx
Allergen Standard:	Recombinant Ara h 8 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	1,000ng/mL (based on amino acid analysis)
Lot Number:	xxxxx

Materials required, but not provided:

- Type I ultrapure water or 18.2MΩ de-ionized water
- Volumetric measuring equipment (e.g. serological pipettes, graduated cylinders)
- Clean containers for buffer and reagent preparation
- Calibrated single and multi-channel micropipettes and tips
- Vortex mixer
- Plate reader capable of reading absorbance at 450nm
- Analysis software (recommended, but not required)

A list of frequently asked questions and troubleshooting guide can be found under the 'Support' tab on our web site: www.inbio.com.

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare a 1x dilution of wash and assay buffers from the 10x concentrates in clean containers using 18.2MΩ de-ionized water or Type I ultrapure water.
   For one plate:
   Wash buffer: add 15mL concentrate to 135mL water (150mL total volume)
   Assay buffer: add 3mL concentrate to 27mL water (30mL total volume)
   Adjust volumes accordingly for multi-plate assays. Diluted buffers may be stored at 4°C for up to 1 week.

   The example below is for testing 6 samples starting at 1/10 dilution. A multichannel pipet is recommended for mixing and transferring between wells.
   - *Highly concentrated samples will require pre-dilution before adding to the plate.*
2. Remove the plate from the foil pouch. Add 150µL wash buffer to each well. Empty the wells by inverting the plate and then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two times.
   *Move directly to the next step to prevent the wells from drying.
3. Add 100µL assay buffer to all wells. Add an additional 80µL of assay buffer to wells A1-H1 (the total volume of assay buffer in these wells will be 180µL; all other wells will have 100µL).
4. **Standard:** gently vortex the Ara h 8 standard and add 20µL to wells A1 and B1. Mix by pipetting up and down 8-10 times, and then transfer 100µL into wells A2 and B2. Mix and continue the doubling dilution scheme across the plate to wells A10 and B10. Remove and discard 100µL from wells A10 and B10 (100µL will remain).
   The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks.**
   Samples: add 20µL of sample to wells C1-H1. Mix by pipetting up and down 8-10 times. Transfer 100µL to wells C2-H2. Continue mixing and transferring to column 12. Remove and discard 100µL from wells C12-H12 (100µL will remain). When finished preparing the plate, the final volume in all wells should be 100µL.
5. Cover the plate and incubate for 1 hour ± 10 minutes at room temperature (20-25°C) away from direct sunlight. **Note:** gentle agitation on a plate shaker during incubations may reduce variability.
6. Wash the plate 3x with 150µL wash buffer per well. Vortex the polyclonal antibody and prepare a 1:1,000 detection antibody mix by adding 11µL polyclonal antibody to 11mL assay buffer. **Mix thoroughly** and add 100µL to each well.
7. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.
8. Wash the plate 3x with 150µL wash buffer per well. Vortex the peroxidase-conjugated goat anti-rabbit IgG and prepare a 1:1,000 conjugate mix by adding 11µL peroxidase-conjugated goat anti-rabbit IgG to 11mL assay buffer. **Mix thoroughly** and add 100µL to each well.
9. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.
10. Pour the TMB substrate and stop solution into separate basins so they are ready to use in the next step. Wash the plate 3x with 150µL wash buffer per well.
11. Use a **multi-channel** pipette to add 100µL TMB to each well. Gently tap the plate and monitor the reaction as the blue color develops. Once OD450 reaches 0.08-0.09 for Standard 1, use a **multi-channel** pipette to add 50µL stop solution to each well (the color will change to yellow).
12. Read the plate at 450nm. The OD for Standard 1 should be between 1.2 and 3.5.