Ara h 2 ELISA kit (1C4/AH2)

Product Code: EL-AH2
Lot Number: xxxxx

Sample Curve:

Content:

Vial 1  (red top) 100 μL
Monoclonal antibody 1C4
Concentration: 2mg/ml in PBS

Vial 2  (white top) 400 μL
Ara h 2 Standard
Concentration: 2500ng/ml Ara h 2

Vial 3  (brown) 100 μL
Rabbit anti Ara h 2 antibody
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.
Certificate of Analysis

Monoclonal Antibody: 1C4 (clone 1C4 G4 C8)
Immunogen: Ara h 2
Isotype: Mouse IgG1
Specificity: Binds to species specific epitope present on Arachis hypogaea allergen, Ara h 2.
Purification: Produced in ascites and purified by affinity chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.
Concentration: 2 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

Antibody: Polyclonal rabbit antiserum raised against natural purified Ara h 2
Specificity: The antiserum contains IgG antibodies to Ara h 2
Activity: The antiserum has been diluted in phosphate buffered saline, pH 7.4, containing 1%BSA/50% glycerol. The antiserum has been 0.22μm filtered and should be diluted 1/1000 for Ara h 2 ELISA.
Lot Number: xxxxx

Allergen Standard: nAra h 2
Composition: Naturally purified Ara h 2 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration: 2500ng/ml
Calibration: The Ara h 2 concentration of the purified Ara h 2 was determined by O.D. 280.
Lot Number xxxxx

ELISA Protocol for Ara h 2.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100μl mAb 1C4 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 2 standard to make a control curve ranging from 250 - 0.5ng/ml Ara h 2: Pipette 20μl Ara h 2 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 2 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 polyclonal Rabbit anti Ara h 2 antibody. 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 Peroxidase conjugated Goat anti-Rabbit IgG (Jackson Laboratories Cat# 111-036-046, reconstituted in 1 ml distilled water and 1ml glycerol). The reconstituted Goat anti-Rabbit IgG should be diluted 1/1000 (i.e. 10μl/10ml) in 1% BSA, PBS-T. Incubate for 1hour 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 2 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.