

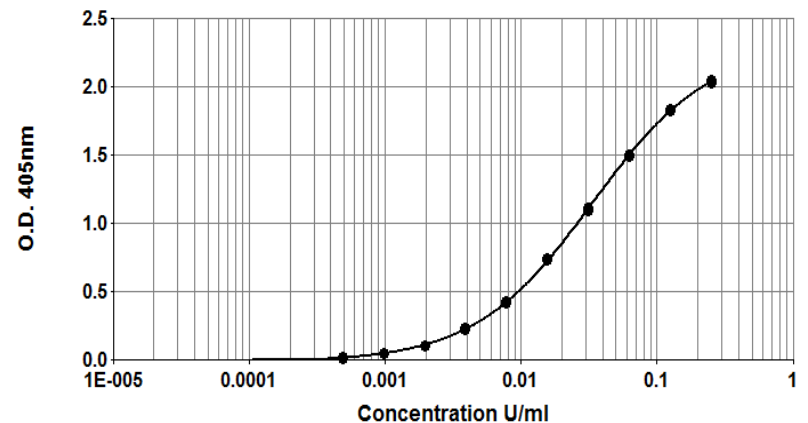


Amb a 1 ELISA kit

Product Code: EL-AM1

Lot Number: xxxxx

Sample Curve:



Content:

- Vial 1 (red top) 100 μ L
Polyclonal rabbit anti-Amb a 1 capture antibody
Concentration: 2mg/ml in PBS
- Vial 2 (white top) 400 μ L
Amb a 1 Standard
Concentration: 2.5 U/ml Amb a 1
- Vial 3 (brown) 100 μ L
Biotinylated rabbit anti-Amb a 1 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.



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Certificate of Analysis

Antibody: Rabbit polyclonal IgG antibodies raised against short ragweed allergen, Amb a 1, and purified by HPLC.
Immunogen: Amb a 1
Isotype: Polyclonal IgG antibodies (isotypes not determined).
Specificity: Binds to *Ambrosia artemisiifolia* allergen, Amb a 1, in short ragweed pollen extracts. Limited cross-reactivity with giant ragweed (*Ambrosia trifida*) extracts.
Undetectable reactivity with grass pollen extracts.
Purification: By HPLC using recombinant protein G. Single heavy and light chain bands on SDS-PAGE.
Concentration: 2.0 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: Rabbit polyclonal IgG antibodies raised against short ragweed allergen, Amb a 1
Immunogen: Amb a 1
Isotype: Polyclonal IgG antibodies (isotypes not determined).
Specificity: Binds to epitopes present on natural ragweed pollen allergen, Amb a 1.
Purification: By affinity chromatography using ragweed antigen.
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: Amb a 1 Standard
Product Code: ST-AM1
Composition: Short Ragweed Extract prepared in 1% BSA/50% glycerol/PBS, 0.22µm filtered, preservative free, pH 7.4
Concentration: 2.5 U/ml Amb a 1
Calibration: Sub-standardized against the U.S. Food and Drug Administration radial immunodiffusion reference for Amb a 1, C14-Ras, which contains 30 Units Amb a 1/ml.
Lot Number: xxxxx

ELISA Protocol for Amb a 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl pAb AM1 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 standard to make a control curve ranging from 0.25 - 0.0005U/ml Amb a 1: Pipette 20µl 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 Amb a 1 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.
4. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Amb a 1 Ab. The antibody solution should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA, PBS-T. Incubate for 1 hour at room temperature.
5. 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 (i.e. 10µl/10ml) in 1% BSA, PBS-T. Incubate for 30 minutes at room temperature.
6. Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H₂O₂ (i.e. 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4, roughly 15-30 minutes.

Notes:

The Amb a 1 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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