

References:

1. Vailes L, Sridhara S, Cromwell O, Weber B, Breitenbach M, Chapman M. Quantification of the major fungal allergens, Alt a 1 and Asp f 1, in commercial allergenic products. *J Allergy Clin Immunol* 2001;107(4):641-6.
2. Unger A, Stoger P, Simon-Nobbe B, Susani M, Cramer R, Ebner C et al. Clinical testing of recombinant allergens of the mold *Alternaria alternata*. *Int Arch Allergy Immunol* 1999;118(2-4):220-1.
3. Asturias JA, Ibarrola I, Ferrer A, Andreu C, Lopez-Pascual E, Quiralte J et al. Diagnosis of *Alternaria alternata* sensitization with natural and recombinant Alt a 1 allergens. *J Allergy Clin Immunol* 2005;115(6):1210-7.
4. Chruszcz M, Chapman MD, Osinski T, Solberg R, Demas M, Porebski PJ et al. *Alternaria alternata* allergen Alt a 1: A unique beta-barrel protein dimer found exclusively in fungi. *J Allergy Clin Immunol* 2012;130(1):241-7.



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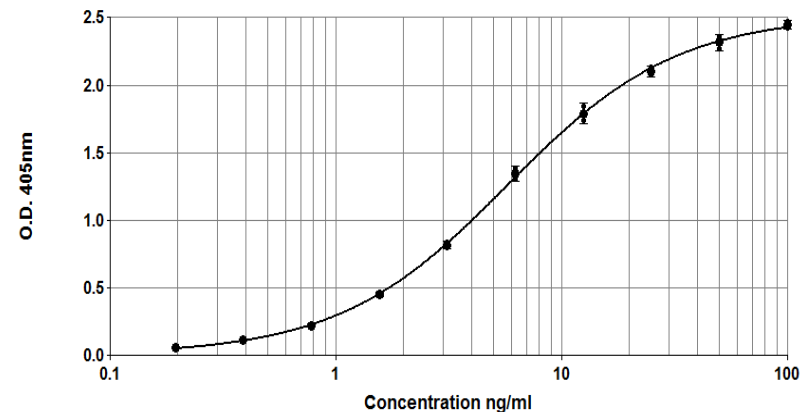


Alt a 1 ELISA kit (2C10/3B6)

Product Code: EL-AA1

Lot Number: XXXXX

Standard Curve:



Contents:

Vial 1 Vial 1 (red top) 100 μ l
Monoclonal antibody 2C10

Vial 2 Vial 2 (white top) 400 μ l
rAlt a 1 Standard
Concentration: 1000ng/ml

Vial 3 Vial 3 (brown) 100 μ l
Biotinylated monoclonal antibody 3B6
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: 2C10 (clone 2C10 G4)
Immunogen: rAlt a 1
Isotype: Mouse IgG
Specificity: Binds to a species specific epitope on *Alternaria alternata* allergen, Alt a 1.
Purification: Produced *in vitro* cell culture and purified by chromatography using recombinant protein G. Single heavy and light chain bands on SDS-PAGE.
Concentration: 1mg/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: 3B6 (clone 3B6 H9)
Immunogen: rAlt a 1
Isotype: Mouse IgG
Specificity: Binds to a species specific epitope on *Alternaria alternata* allergen, Alt a 1.
Purification: Produced *in vitro* cell culture and purified by chromatography using recombinant protein G. Single heavy and light chain bands on SDS-PAGE.
Biotinylation: Biotinylated and titrated for use in ELISA for Alt a 1 allergen at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number: xxxxx

Allergen Standard: rAlt a 1
Composition: Recombinant Alt a 1 prepared in 1% BSA/50% glycerol/PBS. 0.22µm filtered, preservative free, pH 7.4.
Concentration: 1000ng/ml Alt a 1
Calibration: The rAlt a 1 was purified from *Pichia pastoris* by size exclusion HPLC. The protein content was determined by amino acid analysis.
Lot Number: xxxxx

ELISA protocol for Alt a 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100µl mAb 2C10 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 rAlt a 1 Standard to make a control curve ranging from 100 - 0.2ng/ml Alt a 1: Pipette 20µl Alt a 1 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 Alt 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.
5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti - Alt a 1 mAb 3B6. 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 Alt 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.

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