

References:

1. Gill SC, von Hippel PH. Anal Biochem 1989 Nov 1;182(2):319- 26.
2. Vailes L, Sridhara S, Cromwell O, Weber B, Breitenbach M, Chapman M. J Allergy Clin Immunol 2001 Apr;107(4):641-6

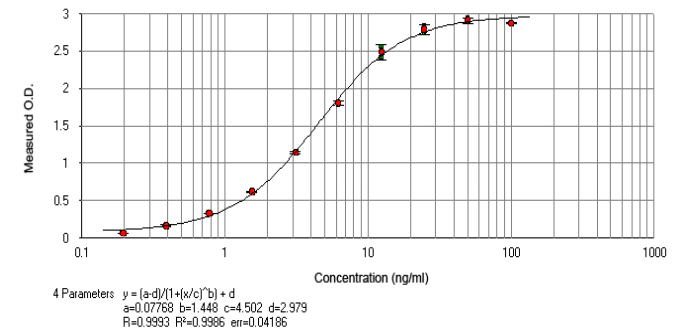


Alt a 1 ELISA kit (121/121) For *Alternaria* Allergen Detection

Product Code: EL-AA1

Lot: xxxxx

Sample Curve:



www.inbio.com

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Content:

- Vial 1 Vial 1 (red top) 0.1ml
Monoclonal antibody 121
Concentration: 2mg/ml in PBS
- Vial 2 Vial 2 (white top) 400 µl
rAlt a 1 Standard
Concentration: 1000ng/ml
- Vial 3 Vial 3 (brown) 100 µl
Biotinylated monoclonal antibody 121
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

ELISA protocol for Alt a 1 allergen.

Certificate of Analysis

Monoclonal Antibody: 121 (clone 121 G5 G8)
Immunogen: Alt a 1
Isotype: Mouse IgG2A
Specificity: Binds to a species specific epitope on Alternaria altanata allergen, Alt a 1.
Purification: From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. Single heavy and light chain bands on SDS-PAGE
Concentration: 2 mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, preservative free.

Lot Number: xxxxx

Monoclonal Antibody: 121 (clone 121 G5 G8)
Immunogen: Alt a 1
Isotype: Mouse IgG1
Specificity: Binds to a species specific epitope on Alternaria altanata allergen, Alt a 1.
Purification: From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. Single heavy and light chain bands on SDS-PAGE
Biotinylation: Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent 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 from E. coli and purified by conventional biochemical methods. The mol.ext.coeff was calculated from the DNA-derived protein sequence, as described by Gill et.al (1).

Lot Number: xxxxx

1. Anti Alt a 1 mAb 121 is supplied HPLC purified and as a stock solution in PBS (Code MA-121). Dilute the mAb 121 1/1000 (i.e. 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog #439454, Fisher Catalog #12565135) with 100 µl of the diluted mAb 121 per well. Incubate overnight 4EC.
2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100µl 1% BSA PBS-T. Wash 3x with PBS-T.
3. Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - 3a. Make a Alt a 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-AA1): The control curve dilutions are 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 of 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, A12 and B12 should contain only 1% BSA PBS-T as blanks.
 - 3b. House dust samples are routinely diluted two-fold from 1/10-1/80.
4. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Alt a 1 mAb 121 (Product Code: BI-121). The antibody solution contains 50% glycerol and 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 1 ml 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.

Notes: *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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