

## References:

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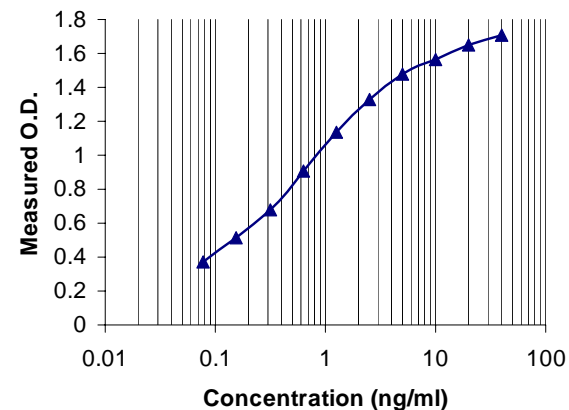
## Asp f 1 ELISA kit For Allergen Detection of *Aspergillus fumigatus*

EL-AF1

Batch: xxxxx

Sample Curve:

Asp f 1 Standard



Content:

- ◆ Vial 1 (red top) 100 µl  
Monoclonal antibody 4A6  
Concentration: 4 mg/ml in PBS
- ◆ Vial 2 (white top) 400 µl  
Asp f 1 Standard  
Concentration: **400ng/ml**
- ◆ Vial 3 (brown) 100 µl  
Polyclonal rabbit anti-Asp f 1 antibody  
Dilute: 1:1000 for use

**Storage: All reagents should be stored at 4°C**

## Certificate of Analysis

Antibody: Monoclonal anti Asp f 1 mAb, 4A6  
Immunogen: Asp f 1  
Isotype: Mouse IgG1  
Specificity: Binds to a common epitope on *Aspergillus fumigatus* allergen, Asp f 1.  
Purification: Produced in tissue culture by hollow fiber fermentation and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE  
Concentration: 2mg/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

Antibody: The rabbit anti-*A.fumigatus* antibody is a polyclonal antiserum raised against partially purified Asp f 1 (a fraction eluted with 2M NaCl from a phenyl-sepharose column) (1, 2).  
Specificity: The antiserum contains IgG antibodies to Asp f 1 as well as IgG antibodies to other *Aspergillus* allergens  
Activity: The antiserum has been diluted in phosphate buffered saline, pH 7.4, containing 1%BSA-50%glycerol.  
The antiserum has been filtered and should be diluted 1/1000 for use in Asp f 1 assays.  
Lot Number: xxxxx

Allergen Standard: Asp f 1  
Composition: *Aspergillus* extract prepared in 1% BSA/50% glycerol/PBS. pH 7.4  
Concentration: 400ng/ml Asp f 1  
Calibration: Asp f 1 was purified from *Aspergillus* extract by affinity chromatography using anti-Asp f 1 monoclonal antibody 4A6. The protein concentration was determined by ELISA.  
Lot Number: xxxxx

## Protocol for Asp f 1 ELISA

1. Anti-Asp f 1 mAb is supplied HPLC purified as a stock solution in PBS (Product Code: MA-4A6). Dilute the mAb 4A6 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 100µl of the diluted mAb 4A6 per well. 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 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 Asp f 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-AF1): The control curve dilutions are from 40-0.08ng/ml Asp f 1. Pipette 20µl Asp f 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 Rabbit anti- Asp f 1 antibody (Product Code: PA-AF1). 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 Peroxidase conjugated Goat anti Rabbit IgG (Jackson Laboratories Cat. #111-036-046, reconstituted in 1ml distilled water and 1ml glycerol). The reconstituted Goat anti Rabbit should be diluted 1/1000 (i.e., 10µl/10ml).
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% H<sub>2</sub>O<sub>2</sub> (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](http://www.inbio.com).

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