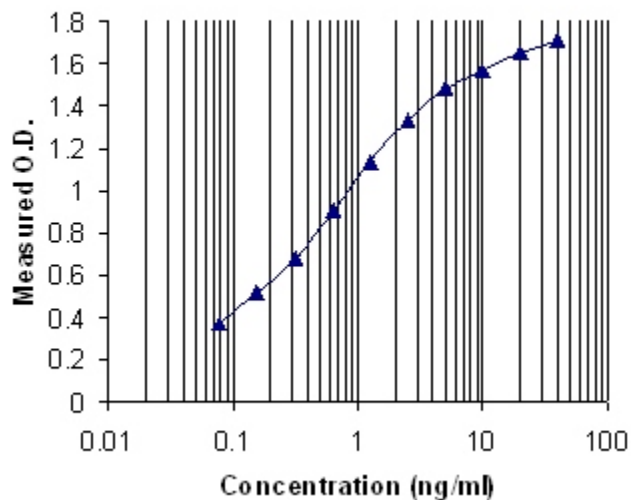




## Asp f 1 ELISA kit

Product Code: EL-AF1

Asp f 1 Standard



### Content:

- ◆ Vial 1 (red top) 100  $\mu$ l  
Monoclonal antibody 4A6  
Concentration: 10mg/ml in PBS
- ◆ Vial 2 (white top) 400  $\mu$ l  
Asp f 1 Standard  
Concentration: **400ng/ml**
- ◆ Vial 3 (brown) 100  $\mu$ l  
Polyclonal rabbit anti-Asp f 1 antibody  
Dilute: 1:1000 for use

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

### Developed and manufactured by:

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### Protocol for Asp f 1 ELISA

1. Anti Asp f 1 mAb 4A6 is supplied SAS purified as a stock solution at 10mg/ml in PBS. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 10 $\mu$ l/10ml of mAb 4A6 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 0.1ml 1% BSA PBS-T. Wash 3x with PBS-T.
3. Use doubling dilutions of the Asp f 1 Standard to make a control curve. The control curve dilutions are from 40-0.04ng/ml Asp f 1. Pipette 20 $\mu$ l Asp f 1 reference into 180 $\mu$ l 1% BSA PBS-T into rows A1 and B1 on the ELISA plate. Mix well and transfer 100 $\mu$ l across the plate into 100 $\mu$ l 1% BSA PBS-T diluent to make 10 serial doubling dilutions. Rows 11 and 12 should contain only 1% BSA PBS-T as blanks.
4. Add 0.1ml of diluted allergen samples and incubate for 1 hour at room temperature. House dust samples are routinely diluted two-fold from 1/10-1/80.
5. Wash wells 3x with PBS-T and add 0.1ml diluted Rabbit anti-Asp f 1. The polyclonal 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 Peroxidase conjugated Goat anti Rabbit IgG 1:1000 (Jackson Laboratories Cat.# 111-036-045 or equivalent supplier). Incubate 1 hour at room temperature.
7. Wash wells 3x and develop the assays by adding 0.1ml 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H<sub>2</sub>O<sub>2</sub>. Read the plate when the absorbance 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).*

**References:**

1. Arruda, LK, Platts-Mills, TAE, Fox, JW and Chapman, MD (1990). *Aspergillus fumigatus* allergen I, a major IgE binding protein, is a member of the mitogillin family of cytotoxins. *J. Exp. Med.*, 172:1529-1532.
2. Arruda LK, Platts-Mills TAE, Longbottom JL, Chapman MD (1992). *Aspergillus fumigatus*: Identification of 16kd, 18kd and 45kd antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. *J. Allergy Clin. Immunol.* 89:1166-1176.
3. Arruda LK, Mann BJ, Chapman MD (1992). Selective expression of a major allergen and cytotoxin, Asp f I, in *Aspergillus fumigatus*. *J. Immunol.*149:3354-3359.
4. Sporik RB, Arruda LK, Woodfolk J, Chapman MD, Platts-Mills TAE. (1993). Environmental exposure to *Aspergillus fumigatus* allergen 1 (Asp f I). *Clin Exp Allergy* 23:326-331.
5. El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TAE, Heymann PW. (1994). Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 150:1513-1518.

**SAMPLE**  
**Certificate of Analysis**  
**(Lot Number subject to change)**

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: 50% saturated ammonium sulfate fraction of ascites, dialyzed against BBS pH 8.0 for 48 hours.  
Concentration: 10mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, preservative free.  
Lot Number: XXXX

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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: XXXX

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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: XXXX