

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 1, 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 1). *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.
6. Vailes LD, Sridhara S, Cromwell O, Weber B, Breitenbach M, Chapman MD (2001). Quantitation of the major fungal allergens, Alt a 1 and Asp f 1, in commercial allergenic products. *J. Allergy Clin. Immunol.* 107:641-646.
7. Chapman MD (2006). Challenges associated with indoor moulds: Health effects, immune response and exposure assessment. *Medical Mycology.* 44:S29-S32.



www.inbio.com

Indoor Biotechnologies, Inc.
1216 Harris Street,
Charlottesville,
VA 22903
United States

Tel: (434) 984-2304
Fax: (434) 984-2709
E-mail: mail@inbio.com

Indoor Biotechnologies Ltd.
Cardiff Medicentre
Heath Park
Cardiff CF14 4UJ
Wales

Tel: +44 (0) 2920 68 2115
Fax: +44 (0) 2920 68 2044
E-mail: info@indoorbiotech.co.uk

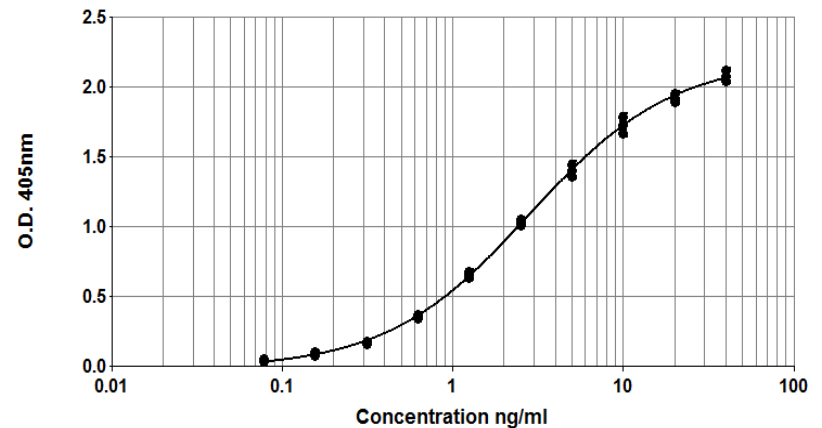


Asp f 1 ELISA kit (4A6/AF1)

Product Code: EL-AF1

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 100 μ L
Monoclonal antibody 4A6
Concentration: 2mg/ml in PBS

Vial 2 (white top) 400 μ L
Asp r 1 Standard
Concentration: 400ng/ml Asp r 1

Vial 3 (brown) 100 μ L
Polyclonal rabbit anti-Asp f 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.

Certificate of Analysis

Monoclonal Antibody: 4A6 (clone 4A6 B5 E7)
Immunogen: Asp f 1
Isotype: Mouse IgG₁
Specificity: Binds to a species specific epitope on *Aspergillus fumigatus* allergen, Asp f 1.
Purification: Produced in tissue culture and purified by chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.
Concentration: 2 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: Polyclonal antibody raised against partially purified Asp f 1 (1,2).
Specificity: The pAb contains IgG Ab to Asp f 1 as well as IgG Ab to other *Aspergillus* allergens.
Activity: The pAb is in phosphate buffered saline, pH 7.4, containing 1% BSA/50% glycerol/PBS. The pAb has been filtered and should be diluted 1/1000 for use in Asp f 1 ELISA.
Lot Number: xxxx

Allergen Standard: nAsp r 1
Composition: Naturally purified Asp r 1 prepared in 1% BSA, 50% glycerol/PBS, pH 7.4
Concentration: 400ng/ml
Calibration: The Asp r 1 concentration of the purified natural Asp r 1 was determined by amino acid analysis. Asp f 1 and Asp r 1 (mitogillin) are 95% homologous (differing by 4 amino acid residues) and are antigenically and functionally indistinguishable (1).
Lot Number: xxxxx

ELISA protocol for Asp f 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 4A6 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 nAsp r 1 standard to make a control curve ranging from 40 - 0.08ng/ml Asp r 1: Pipette 20µl Asp r 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 Asp f 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 polyclonal Rabbit anti Asp f 1 antibody. 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 with PBS-T and add 100µl diluted Peroxidase conjugated Goat anti-Rabbit IgG (Jackson Laboratories Cat# 111-036-046, reconstituted in 1 ml distilled water and 1ml glycerol). The reconstituted Goat anti-Rabbit IgG should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA, PBS-T. Incubate for 1hour 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 Asp r 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.