Assay specificity:

Mold Species

Monoclonal Antibody	*Aspergillus versicolor*	*Stachybotrys chartarum*	*Aspergillus fumigatus*	*Penicillium chrysogenum*	*Penicillium expansum*	*Trichoderma harzianum*	*Chaetomium globosum*
5F5	**1.38**	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11
9A5	**2.26**	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11

Values are O.D. in ELISA (405 nm, assay background <0.11)

INDOOR biotechnologies INC

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

INDOOR biotechnologies INC

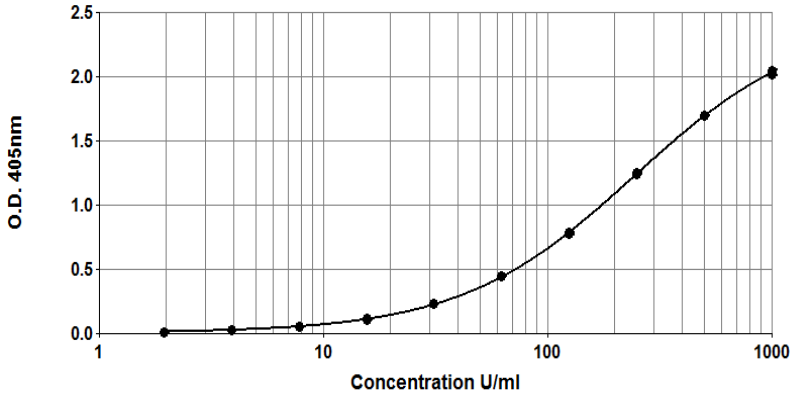
AveX ELISA kit (5F5/9A5)

For *Aspergillus versicolor* Antigen Detection

Product Code: EL-AVX

Lot: XXXXX

Sample Curve:



Content:

Vial 1 (red top) 100 µl
Monoclonal antibody 5F5
Concentration: 1mg/ml in PBS

Vial 2 (white top) 400 µl
Aspergillus versicolor Standard
Concentration: 10,000U/ml

Vial 3 (brown) 100 µl
Biotinylated monoclonal antibody 9A5
Dilute: 1:1000 for use

Storage: All reagents 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:	5F5 (clone 5F5 H2 H8)
Product Code:	MA-5F5
Immunogen:	*Aspergillus versicolor* extract
Isotype:	Mouse IgG2a
Specificity:	Binds to a species specific epitope of *Aspergillus versicolor* antigen.
Purification:	Produced in tissue culture and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration:	1 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**

Monoclonal Antibody:	9A5 (clone 9A5 B9 C10)
Product Code:	BI-9A5
Immunogen:	*Aspergillus versicolor* extract
Isotype:	Mouse IgG2a
Specificity:	Binds to a species specific epitope of *Aspergillus versicolor* antigen.
Purification:	Produced in tissue culture and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Biotinylation:	Biotinylated and titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number:	**XXXXX**

Antigen Standard:	*Aspergillus versicolor*
Product Code:	ST-AVX
Composition:	*Aspergillus versicolor* source material extract prepared in 1% BSA/50% glycerol/PBS, 0.22µm filtered, preservative free, pH 7.4
Concentration:	10,000U/ml AveX
Calibration:	The concentration of the extract was measured using a total protein assay. The extract is calibrated in arbitrary units where 1 unit of AveX equals approximately 1 nanogram of total protein.
Lot Number	**XXXXX**

ELISA protocol for AveX antigen.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 5F5 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 antigen standard to make a control curve ranging from 1000 - 2U/ml AveX: Pipette 20µl of the standard into180µl 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. Environmental samples for AveX analysis are routinely diluted two-fold from 1/2-1/8 for dust and air filter extracts or 1/10-1/80 for swabs, wallboard, and culture extracts.

5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-AveX mAb 9A5. 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.

6. 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.

7. 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_2O_2 (i.e. 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.

Notes:

The antigen 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.