#### Mold Species

	Stachybotrys chartarum	Aspergillus versicolor	Aspergillus fumigatus	Penicillium chrysogenum	Penicillium expansum	Trichoderma harzianum	Chaetomium globosum
3E11	1.77	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12
2C9	2.20	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12

Values are O.D. in ELISA (405 nm, assay background <0.09)



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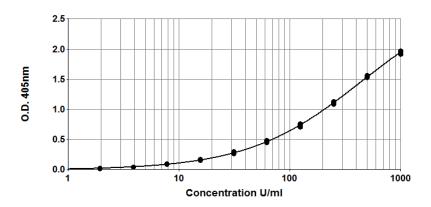


# SchX ELISA kit (3E11/2C9)

# For Stachybotrys chartarum Antigen Detection Product Code: EL-SCX

Lot: XXXXX

## Sample Curve:



#### Content:

Vial 1 (red top) 100 µl

Monoclonal antibody 3E11 Concentration: 1mg/ml in PBS

Vial 2 (white top) 400 µl

Stachybotrys chartarum Standard Concentration: 10.000U/ml

Vial 3 (brown) 100 µl

Biotinylated monoclonal antibody 2C9

Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

# **Certificate of Analysis**

Monoclonal Antibody: 3E11 (clone 3E11 C7 E2)

Product Code: MA-3E11

Immunogen: Stachybotrys chartarum extract

Isotype: Mouse IgG1

Specificity: Binds to a species specific epitope of *Stachybotrys* 

chartarum 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: 2C9 (clone 2C9 E3 D8)

Product Code: BI-2C9

Immunogen: Stachybotrys chartarum extract

Isotype: Mouse IgG1

Specificity: Binds to a species specific epitope of *Stachybotrys* 

chartarum 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: Stachybotrys chartarum

Product Code: ST-SC1

Composition: Stachybotrys chartarum culture extract prepared in

1% BSA/50% glycerol/PBS, 0.22µm filtered,

preservative free, pH 7.4

Concentration: 10,000U/ml SchX

Calibration: The concentration of the extract was measured

using a total protein assay. The extract is calibrated in arbitrary units where 1 unit of SchX

equals approximately 1 nanogram of total protein.

Lot Number XXXXX

## **ELISA** protocol for SchX antigen.

- Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100μl mAb 3E11 at 10μl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.
- 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 SchX: 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 SchX 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.
- Wash wells 3x with PBS-T and add 100μl diluted biotinylated anti-SchX mAb 2C9. 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 assay by adding  $100\mu l$  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.  $10\mu l/10ml$  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.