

Assay specificity:

Monoclonal Antibody

| | Mold Species | | | | | | |
|------|-------------------------------|-------------------------------|------------------------------|--------------------------------|-----------------------------|------------------------------|----------------------------|
| | <i>Stachybotrys chartarum</i> | <i>Aspergillus versicolor</i> | <i>Aspergillus fumigatus</i> | <i>Penicillium chrysogenum</i> | <i>Penicillium expansum</i> | <i>Trichoderma harzianum</i> | <i>Chaetomium globosum</i> |
| 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)



SchX ELISA kit (3E11/2C9)

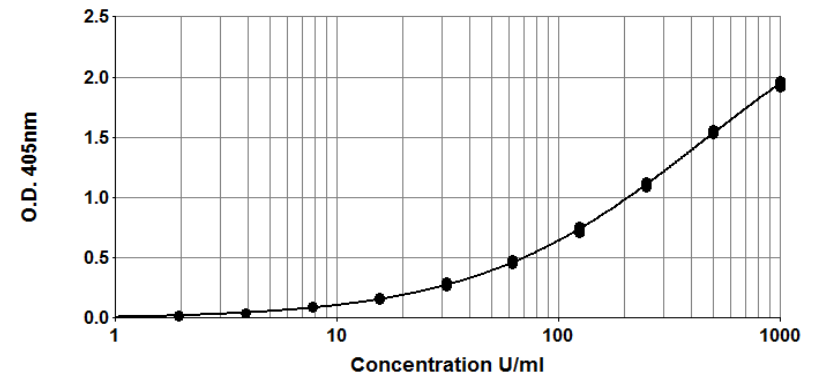
For *Stachybotrys chartarum*

Antigen Detection

Product Code: EL-SCX

Lot: XXXXX

Sample Curve:



Content:

- Vial 1 (red top) 100 μ l
Monoclonal antibody 3E11
Concentration: 1mg/ml in PBS
- Vial 2 (white top) 400 μ l
Stachybotrys chartarum Standard
Concentration: 10,000U/ml
- Vial 3 (brown) 100 μ l
Biotinylated monoclonal antibody 2C9
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.



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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.

1. 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.
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 SchX: Pipette 20µl of the 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. 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.
5. 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 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H₂O₂ (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.