

References:

1. Arruda LK, Rizzo MC, Chapman MD, Fernandez-Caldas E, Baggio D, Platts-Mills TAE, Naspitz CK. Exposure and sensitization to dust mite allergens among asthmatic children in Sno Paulo, Brazil. *Clinical Exp Allergy* 1991; 21:433-439.
2. Vailes LD, Rizzo MC, Naspitz CK, Montealegre F, Fernandez-Caldas E, Platts-Mills TAE, Chapman MD, Arruda LK. Analysis of IgE antibody responses to *D. pteronyssinus* and *Blomia tropicalis* using recombinant group 5 allergens. *J Allergy Clin Immunol* 1996;97:213(abstract).
3. Arruda LK, Vailes LD, Platts-Mills TAE, Fernandez-Caldas E, Montealegre F, Lin KL, Chua KY, Rizzo MC, Naspitz CK, Chapman MD. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Amer J Resp Crit Care Med* 1997; 155:343-350.

Developed and manufactured by:



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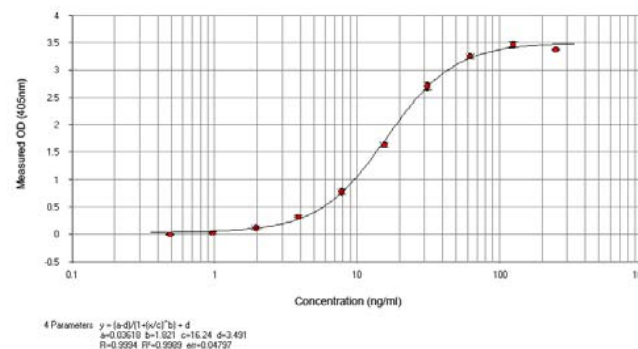


Blo t 5 ELISA kit (4G9/4D9) For Detection of *Blomia tropicalis*

Product Code: EL-BT5

Lot: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 2.0ml
Monoclonal antibody 4G9
Concentration: 5mg/ml in PBS

Vial 2 (white top) 400 μ l
rBlo t 5 Standard
Concentration: 2500ng/ml

Vial 3 (brown) 100 μ l
Biotinylated monoclonal antibody 4D9
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

Certificate of Analysis

Monoclonal Antibody: 4G9 (clone 4G9 A12)
Product Code: MA-4G9
Immunogen: Blo t 5
Isotype: Mouse IgG2A
Specificity: Binds to a species specific epitope on Blomia tropicalis allergen, Blo t 5.
Purification: From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. Single heavy and light chain bands on SDS-PAGE
Concentration: 5.0mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml)
0.22µm filtered, preservative free.
Lot Number: xxxxx

Monoclonal Antibody: 4D9 (clone 4D9 G12 D5A5)
Product Code: BI-4D9
Immunogen: Blo t 5
Isotype: Mouse IgG1
Specificity: Binds to a species specific epitope on Blomia tropicalis allergen, Blo t 5.
Purification: From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. Single heavy and light chain bands on SDS-PAGE
Biotinylation: Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent and titrated for use in ELISA for Blo t 5 allergen at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number: xxxxx

Allergen Standard: rBlo t 5
Product Code: ST-BT5
Composition: Recombinant Blo t 5 prepared in 1% BSA/50% glycerol/PBS. 0.22µm filtered, preservative free, pH 7.4
Concentration: 2500ng/ml rBlo t 5
Calibration: The rBlo t 5 was expressed as a GST-fusion protein, purified over Glutathione Sepharose and further purified trypsin after cleavage by gel filtration HPLC. The protein concentration was determined by Bradford assay.
Lot Number xxxxx

ELISA protocol for Blo t 5 allergen

1. Anti Blo t 5 mAb 4G9 is supplied HPLC purified as a stock solution at 5mg/ml in PBS. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100 µl mAb 4G9 at 200µl/10ml, i.e. 1/50 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, 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 rBlo t 5 Standard to make a control curve. The control curve dilutions are from 250 - 0.5ng/ml Blo t 5. Pipette 20µl Blo t 5 reference into 180µl 1% BSA PBS-T into rows 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. 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 for Blo t 5 analysis are routinely diluted two-fold from 1/5-1/40.
5. Wash wells 3x with PBS-T and add 0.1ml diluted biotinylated anti Blo t 5 mAb 4D9. 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 and add 0.1ml diluted Streptavidin - Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The Streptavidin should be diluted 1/1000 in 1% BSA PBS-T. Incubate for 30 minutes 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 H₂O₂. 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.*

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