

## References:

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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.



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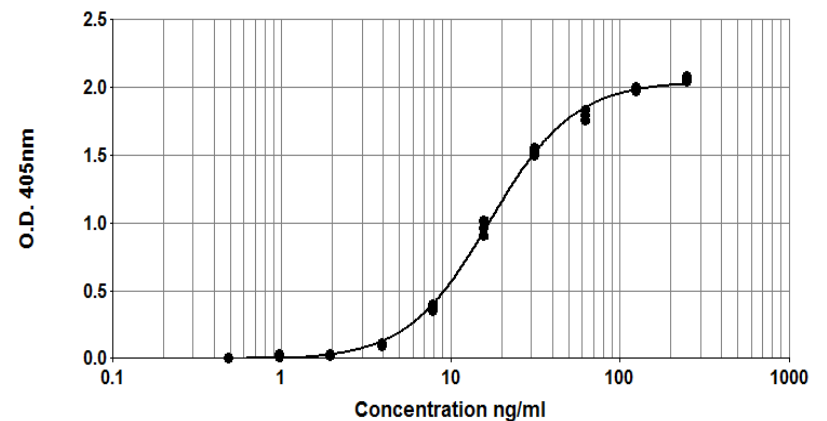


## Blo t 5 ELISA kit (4D9/4G9)

Product Code: EL-BT5

Lot xxxxx

### Sample Curve:



### Content:

Vial 1 (red top) 100µl  
Monoclonal antibody 4D9  
Concentration: 3mg/ml in PBS

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

Vial 3 (brown) 100µl  
Biotinylated monoclonal antibody 4G9  
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:	4D9 (clone 4D9 G12)
Immunogen:	Blo t 5
Isotype:	Mouse IgG1
Specificity:	Binds to a species specific epitope on <i>Blomia tropicalis</i> 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:	3mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42=1mg/ml) 0.22µm filtered, preservative free.
<b>Lot Number:</b>	<b>xxxxx</b>
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Monoclonal Antibody:	4G9 (clone 4G9 A12 D5A5)
Immunogen:	Blo t 5
Isotype:	Mouse IgG2A
Specificity:	Binds to a species specific epitope on <i>Blomia tropicalis</i> 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 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.
<b>Lot Number:</b>	<b>xxxxx</b>
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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 after trypsin cleavage by gel filtration HPLC. The protein concentration was determined by Amino Acid Analysis.
<b>Lot Number</b>	<b>xxxxx</b>

## ELISA protocol for Blo t 5 allergen.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 4D9 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 rBlo t 5 Standard to make a control curve ranging from 250 - 0.5ng/ml Blo t 5: Pipette 20µl Blo t 5 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 Blo t 5 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 biotinylated anti Blo t 5 mAb 4G9. 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 100µl diluted Streptavidin - Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The Streptavidin Peroxidase 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 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H<sub>2</sub>O<sub>2</sub>. 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](http://www.inbio.com).*

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