References:


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

**Product Code: EL-BT5**

**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 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: 3mg/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

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 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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