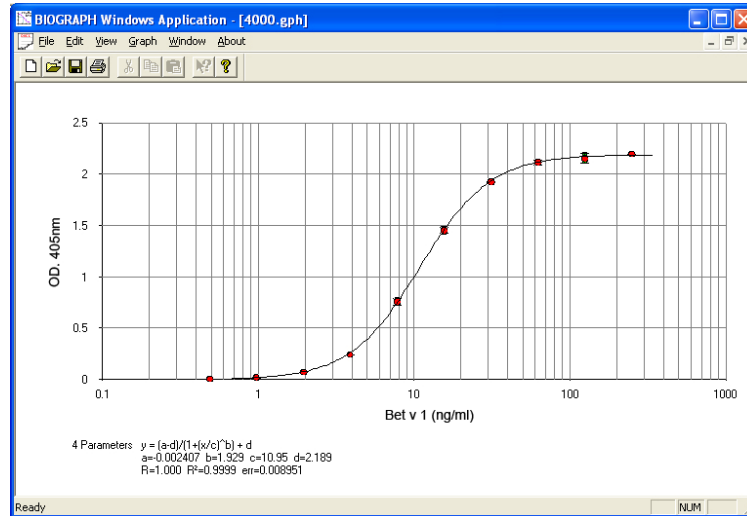




## Bet v 1 ELISA kit (3B4/2E10)

Product Code: EL-BV1

Lot Number: XXXX



### Content:

- ◆ Vial 1 (red top) 100  $\mu$ l  
Monoclonal antibody 3B4  
Concentration 2mg/ml in PBS
- ◆ Vial 2 (white top) 400  $\mu$ l  
rBet v 1 Standard  
Concentration: 2500ng/ml
- ◆ Vial 3 (brown) 100  $\mu$ l  
Biotinylated monoclonal antibody 2E10  
Dilute: 1:1000 for use

**Storage: All reagents should be stored at 4°C**

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**For Research Use Only: Not for Diagnostic or Therapeutic Use**

### ELISA protocol for Bet v 1

1. Anti-Bet v 1 mAb 3B4 is supplied HPLC purified as a stock solution in PBS (Product Code: MA-3B4). Dilute the mAb 3B4 1/1000 (i.e. 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100µl of the diluted mAb 3B4 per well. 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 1% BSA PBS-T. Wash 3x with PBS-T.
3. Add 100µl of diluted allergen standard, allergen extract or air filter samples. Incubate for 1 hour at room temperature.

Make an rBet v 1a control curve using doubling dilutions of the allergen standard (Product Code: ST-BV1): The control curve dilutions are from 250 - 0.5ng/ml rBet v 1a. Pipette 20µl rBet v 1a standard into 180µl 1% BSA PBS-T into wells A1 and B1 of 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, A12 and B12 should contain only 1% BSA PBS-T as blanks.

4. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Bet v 1 mAb 2E10 (Product Code: BI-2E10). 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.
5. Wash wells 3x with PBS-T and add 100µl diluted Streptavidin - Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml 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.
6. Wash wells 3x with PBS-T and develop the assays by adding 100µl 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. 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.

**Notes:** *Buffer recipes, storage conditions and a list of frequently asked questions can be found on the Tech Support link of our web site: [www.inbio.com](http://www.inbio.com).*

**REFERENCES:**

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4. Schappi GF, Monn C, Wuthrich B, Wanner HU. Direct determination of allergens in ambient aerosols: methodological aspects. *Int Arch Allergy Immunol.* 1996 Aug;110(4):364-70.
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**SAMPLE**  
**Certificate of Analysis**  
**(Lot Number subject to change)**

Monoclonal Antibody: 3B4 (clone 3B4F11 D6)  
Immunogen: Bet v 1  
Isotype: Mouse IgG1  
Specificity: Binds to a specific epitope on *Betula verrucosa* allergen, Bet v 1.  
Concentration: 2mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42=1mg/ml) 0.22µm filtered, preservative free.  
Lot Number: XXXX

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Monoclonal Antibody: 2E10 (clone 2E10 G6 G7)  
Immunogen: Bet v 1  
Isotype: Mouse IgG1  
Specificity: Binds to a non-overlapping epitope on *Betula verrucosa* allergen, Bet v 1.  
Biotinylation: Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent and titrated for use in ELISA for Bet v 1 allergen at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered preservative free.  
Lot Number: XXXX

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Allergen Standard: rBet v 1a  
Composition: Recombinant Bet v 1a prepared in 1% BSA/50% glycerol/PBS.  
0.22µm filtered, preservative free, pH 7.4  
Concentration: 2500ng/ml Bet v 1  
Calibration: The rBet v 1a was from *E. coli* and purified by Hydrophobic Interaction Chromatography and Ion Exchange.  
Lot Number: XXXX