

References:

1. Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J.* 1989;8:1935-8.
2. Hoffmann-Sommergruber K, Susani M, Ferreira F, et al. High-level expression and purification of the major birch pollen allergen, Bet v 1. *Protein Expr Purif.* 1997;9:33-9.
3. Godnic-Cvar J, Susani M, Breiteneder H, et al. Recombinant Bet v 1, the major birch pollen allergen, induces hypersensitivity reactions equal to those induced by natural Bet v 1 in the airways of patients allergic to tree pollen. *J Allergy Clin Immunol.* 1997;99:354-9.
4. Schappi GF, Monn C, Wuthrich B, Wanner HU. Direct determination of allergens in ambient aerosols: methodological aspects. *Int Arch Allergy Immunol.* 1996;110:364-70.
5. Schappi GF, Suphioglu C, Taylor PE, Knox RB. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J Allergy Clin Immunol.* 1997;100:656-61.
6. S. Vieths, D. Barber, M. Chapman, et al. Establishment of recombinant major allergens Bet v 1 and Phl p 5a as Ph. Eur. Reference standards and validation of ELISA methods for their measurement. *Pharmeuropa Bio&SN* October 2012; 118-13.



Indoor Biotechnologies, Inc.
1216 Harris Street,
Charlottesville,
VA 22903
United States

Tel: (434) 984-2304
Fax: (434) 984-2709
E-mail: mail@inbio.com

Indoor Biotechnologies Ltd.
Cardiff Medicentre
Heath Park
Cardiff CF14 4UJ
Wales, United Kingdom

Tel: +44 (0) 2920 68 2115
Fax: +44 (0) 2920 68 2044
E-mail: info@indoorbiotech.co.uk

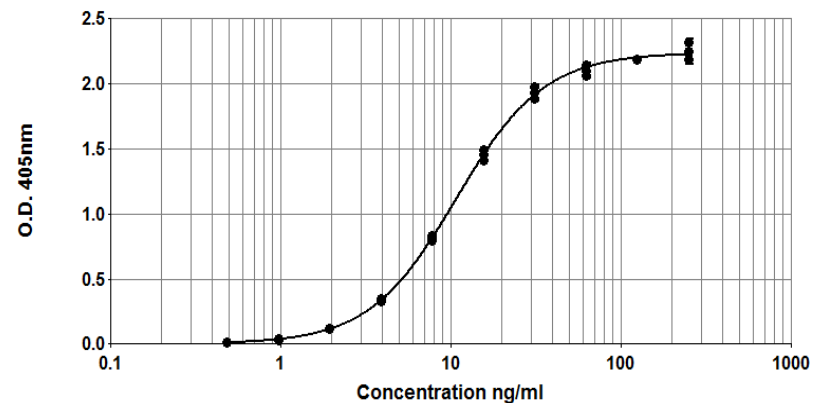


Bet v 1 ELISA kit (4B10/2E10)

Product Code: EL-BV1

Lot xxxxx

Sample Curve:



Content:

Vial 1 (red top) 100 µl
Monoclonal antibody 4B10
Concentration: 1mg/ml in PBS

Vial 2 (white top) 400 µl
rBet v 1 Standard
Concentration: 2500ng/ml

Vial 3 (brown) 100 µl
Biotinylated monoclonal antibody 2E10
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: 4B10 (clone 4B10 D10 F8)
Immunogen: Birch pollen extract
Isotype: Mouse IgG1
Specificity: Binds to multiple isoforms of *Betula verrucosa* allergen, Bet v 1.
Purification: Produced in tissue culture and purified by affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration: 1mg/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: 2E10 (clone 2E10 G6 G7)
Immunogen: Birch pollen extract
Isotype: Mouse IgG1
Specificity: Binds to a non-overlapping, conserved epitope on Bet v 1.
Purification: From tissue culture by ammonium sulphate precipitation and purified by affinity 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

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 produced from *Pichia* and purified by a multi-step chromatography procedure. Validity of the rBet v 1 standard was confirmed by comparison with the European Pharmacopoeia reference CRS (Y0001565), recombinant major allergen rBet v 1 containing 10.25µg of rBet v 1 per vial.
Lot Number xxxxx

ELISA protocol for Bet v 1

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 4B10 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 rBet v 1 standard to make a control curve ranging from 250 - 0.5ng/ml Bet v 1: Pipette 20µl Bet v 1 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 Bet v 1 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-Bet v 1 mAb 2E10. 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 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 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:

The Bet v 1 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.