References:

1. Müller WD, Diener C, Jung K, Jäger L. Antigens of Timothy and other grass pollen extracts identified by monoclonal antibodies. Allergol Immunopathol (Madr). 1988 Sep-Oct;16(5):315-20.
2. Fahlbusch B, Müller WD, Diener CH, Jäger L. Detection of crossreactive determinants in grass pollen extracts using monoclonal antibodies against group IV and group V allergens. Clin Exp Allergy. 1993 Jan;23(1):51-60.
3. Petersen A, Becker WM, Schlaak M. Epitope analysis of isoforms of the major allergen Phl p V by fingerprinting and microsequencing. Clin Exp Allergy. 1994 Mar;24(3):250-6.

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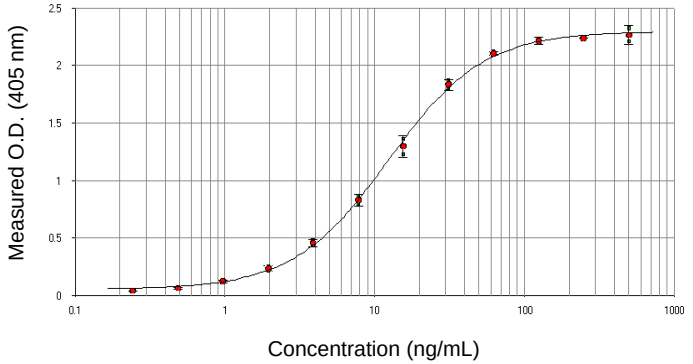
Phl p 5 ELISA kit (1D11/Bo1)

Product Code: EL-PP5

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 1D11
Concentration: 2mg/ml in PBS

Vial 2 (white top) 400 µL
Phl p 5 Standard
Concentration: 5000ng/ml rPhl p 5a

Vial 3 (brown) 100 µL
Biotinylated monoclonal antibody Bo1
Dilute: 1:1000 for use

Storage: The ELISA kit should be stored at 4°C

For Research Use Only: Not for Diagnostic or Therapeutic Use

Certificate of Analysis

Monoclonal Antibody:	1D11 (clone 1D11 C8)
Product Code:	MA-1D11
Immunogen:	Timothy pollen extract
Isotype:	Mouse IgG1
Specificity:	Binds to species specific epitope present on Timothy Grass Pollen Allergen , Phl p 5a & b.
Purification:	Produced in tissue culture and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration:	2.0 mg/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:	Bo 1
Product Code:	BI-BO1
Immunogen:	Crude timothy pollen extract
Isotype:	Mouse IgG1
Specificity:	Binds to species specific epitope present on Timothy Grass Pollen Allergen , Phl p 5a & b.
Purification:	Produced in tissue culture and purified by chromatography using Protein A. 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 Phl p 5 allergen at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, 0.22µm filtered, preservative free.
Lot Number:	**xxxxx**

Allergen Standard:	Phl p 5 Standard
Product Code:	ST-PP5
Composition:	Recombinant Phl p 5a prepared in 1% BSA/50% glycerol/ PBS. 0.22µm filtered, preservative free, pH 7.4
Concentration:	5000ng/ml rPhl p 5a
Calibration:	The rPhl p 5a was from E. coli and purified by conventional biochemical methods. The mol.ext.coeff was calculated from the DNA-derived protein sequence, as described by Genbank acc. No. AJ555152.
Lot Number:	**xxxxx**

ELISA Protocol for Phl p 5.

1. Anti Phl p 5 mAb 1D11 is supplied HPLC purified as a stock solution at 2mg/ml in PBS. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 10µl/10ml of mAb 1D11 in 50mM carbonate-bicarbonate buffer, pH 9.6. Incubate over night at 4^{o}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 Phl p 5 standard to make a control curve. The control curve dilutions are from 500 - 1ng/ml Phl p 5. Pipette 20µl Phl p 5 standard 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 100µl of diluted allergen samples to other rows on the plate and incubate for 1 hour at room temperature. House dust samples are routinely diluted two-fold from1/10-1/80.

5. Wash wells 3x with PBS-T and add 0.1ml diluted biotinylated anti Phl p 5 mAb Bo 1. The antibody solution contains 50% glycerol and should be diluted 1/1000 in 1%BSA PBS-T. Incubate for 1 hour at room temperature. Wash wells 3x and add 100µl 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.

6. Wash wells 3x and develop the assays by adding 0.1ml 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of H_2O_2 (i.e. 1:L of 30% H_2O_2 solution per ml 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 under "Protocols" on our web site: www.inbio.com.