Assay Performance Characteristics:

Standard range: 250 - 0.49ng/mL
Limit of Detection: 0.98ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

Plate Template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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;16:315-20.
2. S. Vieths, D. Barber, M. Chapman, A. Costanzo, A. Daas, H. Fiebig, K.M. Hanschmann, M. Hrabina, S. Kaul, A. Ledesma, P. Moingeon, G. Reese, C. Schörner, R. van Ree, B. Weber, K.H. Buchheit. 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.

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

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

Indoor Biotechnologies Ltd.
Vision Court
Caxton Place
Cardiff, Wales CF23 8HA
United Kingdom

Tel: +44 (0) 29 2167 4640
Email: Info@indoorbiotech.co.uk

Indoor Biotechnologies,
India Private Limited
Bangalore Bioinnovation Centre,
BioTech Park, Electronic City Phase 1
Bangalore-560100, India

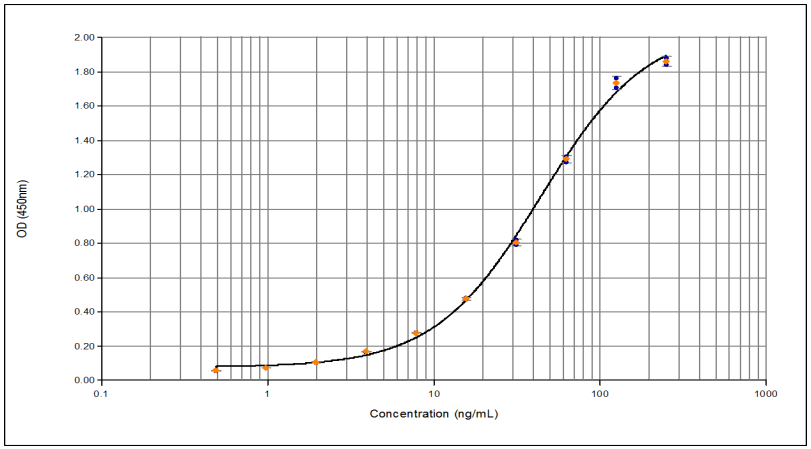
Tel: +91-9901722009
Email: info@inbioindia.com

Phl p 5 ELISA 2.0

Pre-coated Plate Kit

Product Code: EPC-PP5-X
Lot Number: xxxxx

Sample curve:



Contents:
Microtiter plate coated with anti-Phl p 5 monoclonal antibody 1D11

Phl p 5 allergen standard (white cap)
Concentration: 2,500ng/mL

Biotinylated monoclonal antibody Bo1 (brown cap)

Streptavidin-peroxidase (blue cap)

Wash buffer (10x concentrate)
Assay buffer (10x concentrate)
TMB developing substrate
Stop solution (0.5N sulfuric acid)

Store kit at 2-8°C
Expiry:

For research and commercial use in vitro: not for human in vivo or therapeutic use.

An InBio™ product

Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody 1D11 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
Monoclonal Antibody:	1D11 (clone 1D11 C8)
Immunogen:	Timothy grass 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 affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Lot Number:	xxxxx
Detection Antibody:	Bo1
Immunogen:	Crude Timothy grass pollen extract
Isotype:	Mouse IgG1
Specificity:	Binds to species specific epitope present on Timothy Grass Pollen Allergen, Phl p 5a & b.
Purification:	Produced in ascites 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:	Purified recombinant Phl p 5a prepared in 1% BSA/50% glycerol/PBS, pH 7.4. Preservative-free.
Concentration:	2,500 ng/mL
Calibration:	The rPhl p 5a was produced in E. coli and purified by conventional biochemical methods. Validity of the rPhl p 5 standard was confirmed by comparison with the European Pharmacopoeia reference CRS (Y0001566), recombinant major allergen rPhl p 5 containing 8.56 µg of rPhl p 5 per vial.
Lot Number:	xxxxx

Materials required, but not provided:

- Type I ultrapure water or 18.2MΩ de-ionized water
- Volumetric measuring equipment (e.g. serological pipettes, graduated cylinders)
- Clean containers for buffer and reagent preparation
- Reagent reservoirs
- Calibrated single and multi-channel micropipettes and tips
- Vortex mixer
- Plate reader capable of reading absorbance at 450nm
- Analysis software (recommended, but not required)

Protocol

Please read the entire protocol before starting the assay
Bring all reagents to room temperature before use

1. Prepare a 1x dilution of the 10x wash and assay buffer concentrates in clean containers using 18.2MΩ de-ionized water or Type I ultrapure water. For one plate:
   Wash buffer: add 15mL concentrate to 135mL water
   Assay buffer: add 3mL concentrate to 27mL water
   Adjust volumes accordingly for multi-plate assays.
   *Diluted buffers may be stored at 4^{O}C for up to 1 week

2. Remove the plate from the foil pouch and wash by adding 150µL wash buffer to each well. Empty the wells by inverting the plate and then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two more times.

3. Add standards, samples, and blanks to the plate.
   - Extracts of dust samples are routinely started at 1/10 dilution. Air filter extracts, allergen extracts, and other types of samples may require a different dilution scheme
   - Standard and sample dilutions can be prepared directly on the plate
   - Pre-dilutions of samples can be made in tubes or on a dilution plate if necessary. A minimum of three dilutions per sample is recommended
   - The example below is for testing six samples starting at 1/10 dilution

   Add 100µL assay buffer to all wells, plus an additional 80µL to wells in column 1.
   Standard: gently vortex the Phl p 5 standard and add 20µL to wells A1 and B1. Mix well by pipetting up and down 7-10 times and then transfer 100µL into wells A2 and B2. Mix and continue the serial doubling dilution scheme across the plate to column 10. The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks.**
   Samples: add 20µL sample to wells C1 through H1. Mix, then transfer 100µL into 100µL assay buffer in the next well. Continue across the plate for the desired number of dilutions.
   *Remove and discard 100µL from the last wells for the standard and sample dilutions (final volume in all wells should be 100µL).

4. Cover the plate and incubate at room temperature (away from direct sunlight) for 1 hour. *Gentle agitation on a plate shaker during incubations may reduce variability.

5. Wash the plate 3x with 150µL wash buffer per well. Gently vortex the biotinylated Bo1 and prepare a 1:1,000 detection antibody/conjugate mix by adding 11µL biotinylated Bo1 and 11µL streptavidin-peroxidase to 11mL assay buffer. **Mix thoroughly** and add 100µL to each well.

6. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.

7. Pour the TMB substrate and stop solution into separate reagent reservoirs so they are ready to use in the next step. Wash the plate 3x with 150µL wash buffer per well.

8. Use a **multi-channel** pipette to add 100µL TMB to each well and monitor the reaction as the blue color develops. Once OD450 reaches 0.08-0.09 for Standard 1, use a **multi-channel** pipette to add 50µL stop solution to each well (the color will change to yellow).

9. Read the plate at 450nm within 30 minutes (absorbance values begin to decrease after 30 minutes). The OD for Standard 1 should be between 1.2 and 3.5.

A list of frequently asked questions and troubleshooting guide can be found under the 'Support' tab on our web site: www.inbio.com.