

References:

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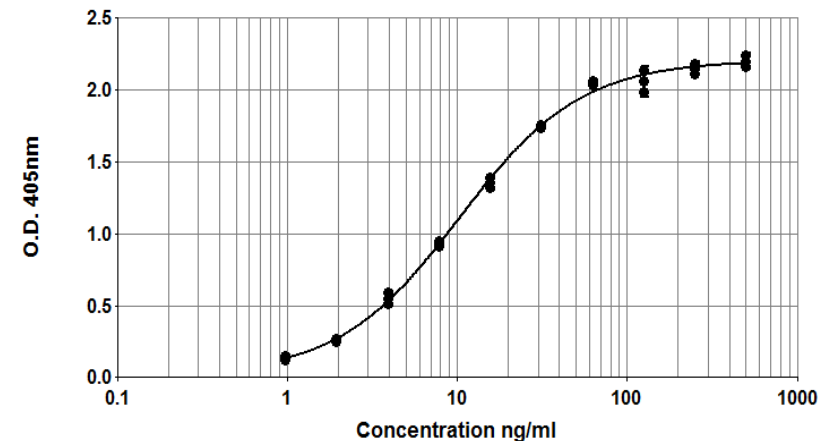


Phl p 5 ELISA kit (1D11/Bo1)

Product Code: EL-PP5

Lot Number: xxxxx

Sample Curve:



Content:

- Vial 1 (red top) 100 μ L
Monoclonal antibody 1D11
Concentration: 1.5mg/ml in PBS
- Vial 2 (white top) 400 μ L
Phl p 5 Standard
Concentration: 5000ng/ml rPhl p 5a
- Vial 3 (brown) 100 μ L
Biotinylated monoclonal antibody Bo 1
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: 1D11 (clone 1D11 C8)
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: 1.5 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
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: 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: rPhl p 5a
Composition: Recombinant Phl p 5a prepared in 1% BSA/50% glycerol/PBS. 0.22µm filtered, preservative free, pH 7.4.
Concentration: 5000ng/ml
Calibration: The rPhl p 5a was from 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

ELISA Protocol for Phl p 5

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 1D11 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 rPhl p 5a standard to make a control curve ranging from 500 - 1ng/ml Phl p 5: Pipette 20µl Phl p 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 Phl p 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 Phl p 5 mAb Bo1. 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:

The Phl p 5 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.

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