

References:

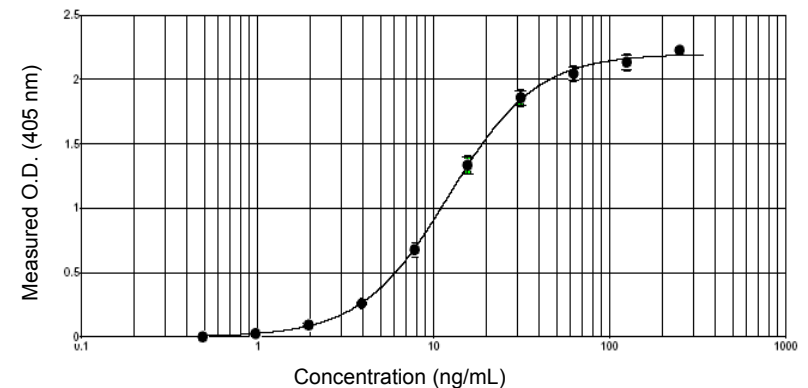
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Der p 1 ELISA kit (10B9/5H8)

Product Code: EL-DP1A
Lot: 31034

Sample Curve:



Developed and manufactured by:



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Content:

Vial 1 (red top) 200 µl
Monoclonal antibody 10B9
Concentration: 1mg/ml in PBS

Vial 2 (white top) 2 x 400 µl
Der p 1 Standard
Concentration: 2500ng/ml

Vial 3 (brown) 200 µl
Biotinylated monoclonal antibody 5H8
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

Certificate of Analysis

Monoclonal Antibody: 10B9
Immunogen: Der p 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on mite Dermatophagoides pteronyssinus allergen, Der p 1.
Purification: Produced in tissue culture by hollow fiber fermentation and purified by chromatography using
Protein A. Single heavy and light chain bands on SDS-PAGE
Concentration: 1.0mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, preservative free.
Lot Number: XXXXX

Monoclonal Antibody: 5H8 (clone 5H8 C12 D8)
Immunogen: Der p 1
Isotype: Mouse IgG2A
Specificity: Binds to a common epitope on mite Dermatophagoides pteronyssinus allergen, Der p 1.
Purification: Produced in tissue culture by hollow fiber fermentation 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 for mite Group 1 allergen at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS.
Lot Number: XXXXX

Allergen Standard: Universal Allergen Standard
Composition: A formulation of eight purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4
Concentration/Calibration:
Lot Number XXXXX

Universal Allergen Standard	Protein Measurement	Concentration (ng/ml)
Der p 1	Amino-acid analysis	2500
Der f 1	Amino-acid analysis	2500
Der p 2	Amino-acid analysis	1000
Fel d 1	Amino-acid analysis	1000
Can f 1	Amino-acid analysis	2500
Rat n 1	Amino-acid analysis	1000
Mus m 1	Amino-acid analysis	250
Bla g 2	Amino-acid analysis	2500

ELISA protocol for Der p 1

- Anti Der p 1 mAb 10B9 is supplied HPLC purified as a stock solution at 1mg/ml in PBS. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 200ng/well mAb 10B9 (i.e. 0.1ml 1/1000 dilution of mAb) in 50mM carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C.
- Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 1 hour with 0.1ml 1% BSA PBS-T then wash 3x with PBS-T and dry.
- Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - Make a Der p 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-DP1): The control curve dilutions are from 250 - 0.5ng/ml Der p 1. Pipette 20µl Der p 1 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.
 - House dust samples are routinely diluted two-fold from 1/10-1/80.
- Wash wells 3x with PBS-T, then incubate for 1 hour with 0.1ml 1/1000 dilution of biotinylated anti Group 1 mAb 5H8.
- Wash wells 3x and incubate for 30 minutes with 0.1ml 1/1000 dilution of Streptavidin - Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The Streptavidin should be diluted in 1% BSA PBS-T. Wash wells 3x and develop the assays by adding 0.1ml 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H₂O₂.
- Read the plate when the absorbance (405nm) reaches 2.0-2.4 or stop the reaction by adding 0.1ml 2mM sodium azide. Absorbance readings are directly proportional to the quantity of Der p 1 bound and values are interpolated from the respective control curves.

Notes: The Der p 1 standard contains 2500ng/ml Der p 1 and has been sub-standardized against the WHO/IUIS D. pteronyssinus reference (NIBSC 82/518), which contains 12.5µg/ml Der p 1(9,10).

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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