

References:

1. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major Dermatophagoides spp. allergens, Der p I and Der f I. J Immunol Methods 1989; 118(2):227-235.
2. Custovic A, Taggart SC, Francis HC, Chapman MD, Woodcock A. Exposure to house dust mite allergens and the clinical activity of asthma. J Allergy Clin Immunol 1996; 98(1):64-72.
3. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997; 100(6 Pt 1):S2-24.
4. Sawyer G, Kemp T, Shaw R et al. Biologic pollution in infant bedding in New Zealand: high allergen exposure during a vulnerable period. J Allergy Clin Immunol 1998; 102(5):765-770.
5. Arbes SJ, Jr., Cohn RD, Yin M et al. House dust mite allergen in US beds: results from the First National Survey of Lead and Allergens in Housing. J Allergy Clin Immunol 2003; 111(2):408-414.
6. Gruchalla RS, Pongracic J, Plaut M et al. Inner City Asthma Study: relationships among sensitivity, allergen exposure, and asthma morbidity. J Allergy Clin Immunol 2005; 115(3):478-485.
7. Zock JP, Heinrich J, Jarvis D et al. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol 2006; 118(3):682-690.
8. Jarvis D, Zock JP, Heinrich J et al. Cat and dust mite allergen levels, specific IgG and IgG(4), and respiratory symptoms in adults. J Allergy Clin Immunol 2007; 119:697-704.
9. Earle CD, King EM, Tsay A et al. High-throughput fluorescent multiplex array for indoor allergen exposure assessment. J Allergy Clin Immunol 2007; 119(2):428-433.



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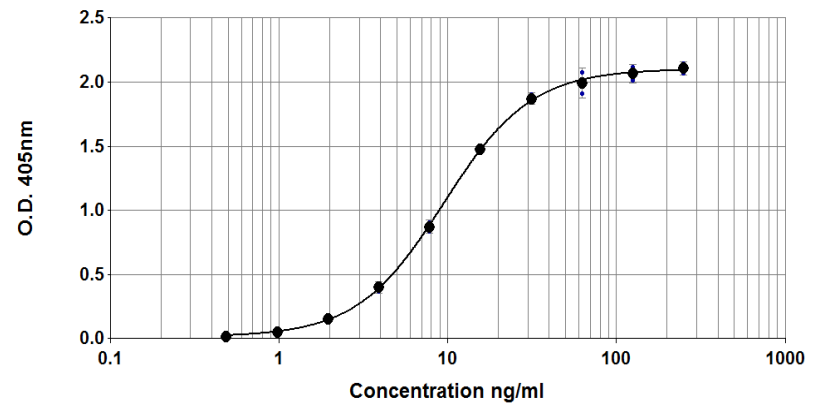


Der p 1 ELISA kit (5H8/4C1)

Product Code: EL-DP1

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 100 μ L
Monoclonal antibody 5H8
Concentration: 2mg/ml in PBS

Vial 2 (white top) 400 μ L
Universal Allergen Standard
Concentration: 2500ng/ml Der p 1

Vial 3 (brown) 100 μ L
Biotinylated monoclonal antibody 4C1
Dilute: 1:1000 for use

Storage: The ELISA kit 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: 5H8 (clone 5H8 C12 D8)
Immunogen: Der p 1
Isotype: Mouse IgG2A
Specificity: Binds to an epitope on dust mite *Dermatophagoides pteronyssinus* allergen, Der p 1.
Purification: Produced in tissue culture and purified by affinity 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=mg/ml), 0.22µm filtered, preservative free.
Lot Number: **xxxxx**

Monoclonal Antibody: 4C1 (clone 4C1 B8 3F8)
Immunogen: Der f 1
Isotype: Mouse IgG1
Specificity: Binds to an epitope on dust mite *Dermatophagoides* Group 1 allergens (Der f 1, Der p 1, Der m 1, Eur m 1).
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: Universal Allergen Standard
Composition: A formulation of eight purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Lot Number: **xxxxx**

Concentration:

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.

- Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 5H8 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.
- 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.
- Use doubling dilutions of the Universal Allergen Standard to make a control curve ranging from 250 - 0.5ng/ml Der p 1: Pipette 20µl UAS 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.
- Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Der p 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.
- Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Der p 1 mAb 4C1. 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 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.
- 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:

Universal Allergen 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.