

## References

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2. Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD. Conformational stability of B cell epitopes on Group I and Group II *Dermatophagoides* spp. allergens. *J Immunol* 1990;144:1353-1360.
3. DeBlay F, Heymann PW, Chapman MD, Platts-Mills TAE. Airborne dust mite allergens: comparison of Group II allergens with Group I mite allergen and cat allergen, Fel d I. *J Allergy Clin Immunol* 1991;88:919-926.
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5. Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor Allergens and Asthma: Report of the Third International Workshop. *J Allergy Clin Immunol*, 1997; 100:S1

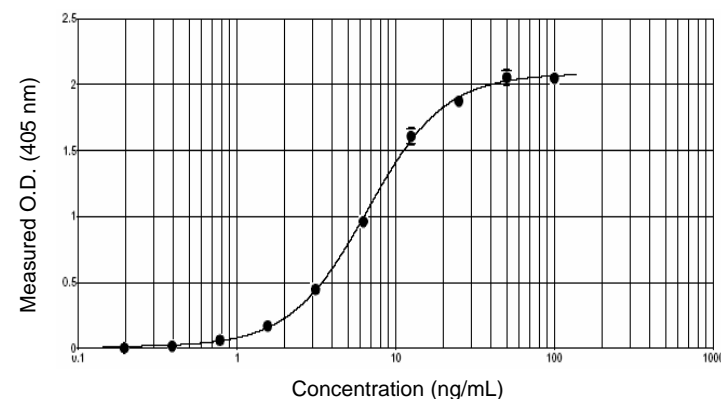


## Mite Group 2 ELISA kit (1D8/7A1)

**Product Code: EL-D2**

**Lot Number: xxxxx**

### Sample Curve:



[www.inbio.com](http://www.inbio.com)

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### Content:

Vial 1 (red top) 100  $\mu$ L  
Monoclonal antibody 1D8  
Concentration: 2mg/ml in PBS

Vial 2 (white top) 400  $\mu$ L  
Universal Allergen Standard  
Concentration: 1000ng/ml Der p 2

Vial 3 (brown) 100  $\mu$ L  
Biotinylated monoclonal antibody 7A1  
Dilute: 1:1000 for use

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

## Certificate of Analysis

**Monoclonal Antibody:** 1D8 (clone 1D8 C4 D8)  
**Immunogen:** Der p 2  
**Isotype:** Mouse IgG1  
**Specificity:** Binds to a common epitope on mite Dermatophagoides spp. Group 2 allergens, Der p 2 and Der f 2.  
**Purification:** From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. 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:** Biotin 7A1 (clone 7A1 H11 G3)  
**Immunogen:** Der f 2  
**Isotype:** Mouse IgG1  
**Specificity:** Binds to a common epitope on mite Dermatophagoides spp Group 2 allergens (Der f 2 and Der p 2).  
**Purification:** From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. 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 mite Group 1 allergen 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  
**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 Mite Group 2 Allergens.

- Anti-Der p 2 mAb 1D8 is supplied HPLC purified as a stock solution at 2mg/ml in PBS (Product Code: MA-1D8). Dilute the mAb 1D8 1/1000 (i.e. 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat poly styrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog #439454, Fisher Catalog #12565135) with 100µl of diluted mAb 1D8 per well. Incubate overnight at 4°C.
- Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30min at room temperature with 100µl 1% BSA-PBS-T. Wash 3x with PBS-T.
- Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
  - Make a Der p 2 control curve using doubling dilutions of the Universal Allergen Standard (Product Code: ST-UAS): The control curve dilutions are from 100-0.2 ng/ml Der p 2. Pipette 20µl allergen 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, and 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 and add 100µl diluted biotinylated anti-Group 2 mAb 7A1 (Product Code: BI-7A1). The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e. 10µl/10ml) 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 (i.e. 10µl/10ml) in 1% BSA PBS-T. Incubate for 30 minutes at room temperature.
- Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H<sub>2</sub>O<sub>2</sub> ( i.e. 10µl/10ml ABTS). Read the plate when the optical density 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](http://www.inbio.com).*

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