

References:

1. Chapman, M.D., Sutherland, W.M., and Platts-Mills, T.A.E. (1984). Recognition of two Dermatophagoides pteronyssinus specific epitopes on Antigen P1 using monoclonal antibodies: Binding to each epitope can be inhibited by sera from mite allergic patients. *J. Immunol.* 133:2488-2495.
2. Ford, A.W., Rawle, F.C., Lind, P., Spieksma, F.Th.M., Lowenstein, H. and Platts-Mills, T.A.E. (1985). Standardization of Dermatophagoides pteronyssinus: Assessment of potency and allergen content in ten coded extracts. *Int. Archs. Allergy Appl. Immunol.* 76:58-68.
3. Heymann, P.W., Chapman, M.D., and Platts-Mills, T.A.E. (1986). Antigen Der p I from the dust mite Dermatophagoides farinae: Structural comparison with Der p I from D. pteronyssinus and epitope specificity of murine IgG and human IgE antibodies. *J. Immunol.* 137:2841-2847.
4. Chapman, M.D., Heymann, P.W., Wilkins, S.R., Brown, M.J., and Platts-Mills, T.A.E. (1987). Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. *J. Allergy Clin. Immunol.* 80:184-194.
5. Platts-Mills, T.A.E. and Chapman, M.D. (1987). Dust mites: Immunology, allergic disease and environmental control. *J. Allergy Clin. Immunol.* 80:755-775.
6. Chapman, M.D., Heymann, P.W., and Platts-Mills, T.A.E. (1987). Epitope mapping of two major inhalant allergens, Der p I and Der f I, from mites of the genus Dermatophagoides. *J. Immunol.* 139:1479-1484.
7. Chapman, M.D. (1988). Allergen specific monoclonal antibodies: New tools for the management of allergic disease. *Allergy* 43 (5):7-14.
8. Luczynska, C.M., Arruda, L.K., Platts-Mills, T.A.E., Miller, J.D., Lopez, M., and Chapman, M.D. (1989). A two-site monoclonal antibody ELISA for the quantification of the major Dermatophagoides spp. allergens, Der p I and Der f I. *J. Immunol. Meths.* 118:227-235.
9. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D and Chapman MD. (1992). Dust mite allergens and asthma: Report of a second international workshop. *J Allergy Clin Immunol* 89:1046-1060.

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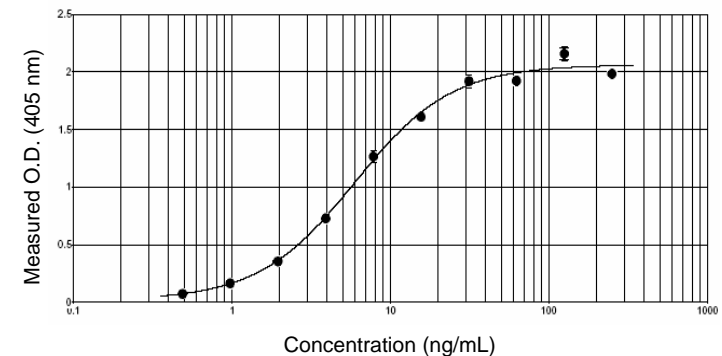


Der f 1 ELISA kit (6A8/4C1)

Product Code: EL-DF1

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 200 µl
Monoclonal antibody 6A8
Concentration: 2mg/ml in PBS

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

Vial 3 (brown) 200 µl
Biotinylated monoclonal antibody 4C1
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

Certificate of Analysis

Monoclonal Antibody: 6A8 (clone 6A8 B10 D12)
Immunogen: Der f 1
Isotype: Mouse IgG1
Specificity: Binds to common epitope present on *Dermatophagoides farinae* allergen, Der f 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: 2.0 mg/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: 4C1 (clone 4C1 B8 3F8)
Immunogen: Der f 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on mite Dermatophagoides Group 1 allergens (Der f 1, Der p 1, Der m 1, Eur m 1).
Purification: By HPLC using recombinant 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 ELISA 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 f 1.

- Anti-Der f 1 mAb 6A8 is supplied HPLC purified as a stock solution at 2mg/ml in PBS (Product Code: MA-6A8). Dilute the mAb 6A8 1/1000 (i.e., 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100µl of the diluted mAb 6A8 per well. 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 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 f 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-DF1): The control curve dilutions are from 250 - 0.5ng/ml Der f 1. Pipette 20µl Der f 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 and add 100µl diluted biotinylated anti-Group 1 mAb 4C1 (Product Code: BI-4C1). 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 1 ml 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% H₂O₂ (i.e., 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.

Notes:

The Der f 1 standard 2500ng/ml Der f 1 and was sub-standardized against the WHO/IUIS D. pteronyssinus reference using a cross-reacting RIA (as yet, there is no International Reference Preparation of D. farinae).

Buffer recipes, storage conditions and a list of frequently asked questions can be found under "Protocols" on our web site: www.inbio.com.