

## References:

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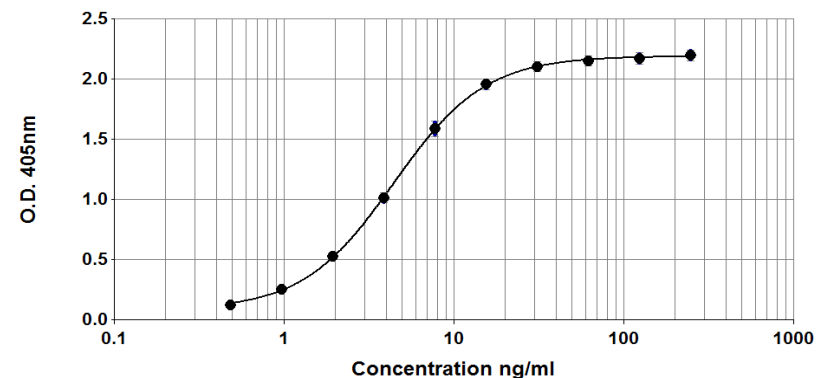


## Der f 1 ELISA kit (6A8/4C1)

**Product Code: EL-DF1**

**Lot Number: xxxxx**

### Sample Curve:



### Content:

Vial 1 (red top) 100  $\mu$ l  
Monoclonal antibody 6A8  
Concentration: 1mg/ml in PBS

Vial 2 (white top) 400  $\mu$ l  
Universal Allergen Standard  
Concentration: 2500ng/ml

Vial 3 (brown) 100  $\mu$ l  
Biotinylated monoclonal antibody 4C1  
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: 6A8 (clone 6A8 B10 D12)  
 Immunogen: Der f 1  
 Isotype: Mouse IgG1  
 Specificity: Binds to a species specific epitope on dust mite *Dermatophagoides farinae* allergen, Der f 1.  
 Purification: Produced in ascites and purified by affinity chromatography using Protein G. Single heavy and light chain bands on SDS-PAGE.  
 Concentration: 1.0 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: 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 G. 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
<b>Der f 1</b>	<b>Amino-acid analysis</b>	<b>2500</b>
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.

- Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 6A8 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 f 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 f 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 f 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 H<sub>2</sub>O<sub>2</sub>. 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](http://www.inbio.com).*

**For research and commercial use in vitro: not for human in vivo or therapeutic use.**