Assay Performance Characteristics:

Standard range: 50-0.1 ng/mL
Limit of Detection: 0.19ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

Plate Template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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 be 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 ex tracts. Int. Archs. Allergy Appl. Immunol. 76:58-68.

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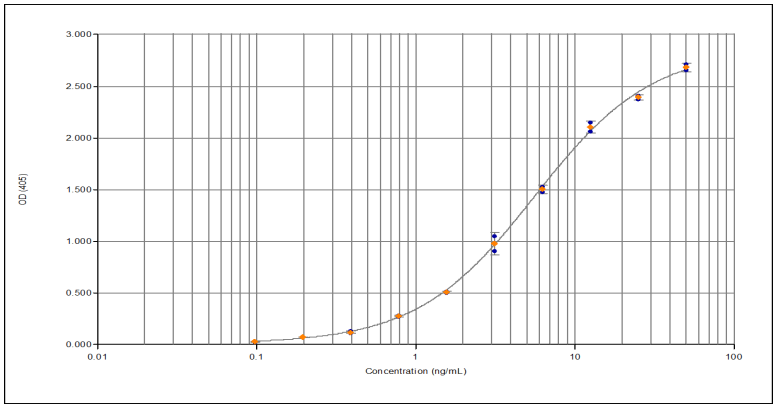
Der f 1 ELISA 2.0

Pre-coated Plate Kit

Product Code: EPC-DF1-X

Lot Number: XXXXX



Contents:

Plate: Pre-coated with anti-Der f 1 monoclonal antibody 6A8

Vial 1: (white top) Der f 1 allergen standard
Concentration: 500 ng/ml

Vial 2: (brown) Biotinylated monoclonal antibody 4C1

Vial 3: (red top) Streptavidin-peroxidase

Bottle 1: Wash buffer, (10x concentrate)
Bottle 2: Assay buffer, (10x concentrate)
Bottle 3: TMB developing substrate
Bottle 4: Stop solution (0.5N sulfuric acid)

Store kit at 2-8^{O}C
Expiry: 6 months from date of receipt

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

Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody 6A8 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
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.
Lot Number:	XXXXX
Detection 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:	Purified natural Der f 1 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	500 ng/mL (based on amino acid analysis)
Lot Number:	XXXXX

Materials required, but not provided:

- Type I ultrapure water or 18.2MΩ de-ionized water
- Volumetric measuring equipment (e.g. serological pipette, graduated cylinder)
- Clean containers for buffer and reagent preparation
- Calibrated single and multi-channel micropipettes and tips
- Vortex mixer
- Plate reader capable of reading absorbance at 450nm
- Analysis software (recommended, but not required)

Protocol

Please read entire protocol before starting the assay
Bring all reagents to room temperature and vortex before use.

1. Allow the pre-coated plate to reach room temperature while in the sealed pouch.

2. Prepare 1x working dilutions of the 10x wash and assay buffers in clean containers using 18.2MΩ de-ionized water or Type I ultrapure water. For one plate:
   Wash buffer: add 15mL concentrate to 135mL water
   Assay buffer: add 2.5mL concentrate to 22.5mL water
   Adjust volumes accordingly for multi-plate assays.
   *Diluted buffers may be stored at 4^{O}C for up to 1 week

3. Remove the plate from the foil pouch and wash by adding 150µL wash buffer to each well. Empty the wells by inverting the plate and tapping on absorbent paper. Repeat the wash 2x.

4. Add standards, samples and blanks to the plate (final volume in all wells should be 100µL).
   Standards: pipette 180µL assay buffer into wells A1 and B1 and 100µL into remaining wells of rows A and B. Add 20µL Der f 1 standard to wells A1 and B1. Mix well and transfer 100µl into wells A2 and B2. Continue across the plate to wells A10 and B10 to make 10 serial doubling dilutions.
   Samples: dust extracts for Der f 1 analysis are routinely diluted two-fold starting at 1/10. Other types of samples, like air filter extracts and allergen extracts, may require different dilutions. It is recommended to test each sample at a minimum of three dilutions; 6-12 are recommended.
   Blanks: add assay buffer to wells A11, B11 and A12, B12.

5. Return the plate to the foil pouch or cover with plate sealer and incubate for 1 hour at room temperature.

6. Wash wells 3x with 150µL wash buffer. Prepare a 1:1,000 detection antibody/conjugate mix by adding 10µL biotinylated 4C1 and 10µL streptavidin-peroxidase to 10mL assay buffer. Mix well and add 100µl to each well. Cover and incubate for 1 hour at room temperature.

7. Wash wells 3x with 150µL wash buffer. Add 100µl TMB substrate to each well and monitor the reaction as the blue color develops. When OD450nm reaches 0.08 for the first standard in wells A1 and B1 (generally within 1-5 minutes), add 50µL stop solution (the color will change to yellow).

8. Read the plate at 450nm. The ideal OD for standard 1 is 2.0-2.5.

Notes:
The *allergen standard is recommended for immunoassay calibration purposes only.*

A list of frequently asked questions and troubleshooting guide can be found under the 'Support' tab on our web site: www.inbio.com.