

Assay Performance Characteristics:

Standard range: 10-0.02ng/mL
Limit of Detection: 0.02ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

References:

1. 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.
2. Platts-Mills TA, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357(9258):752-756.



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

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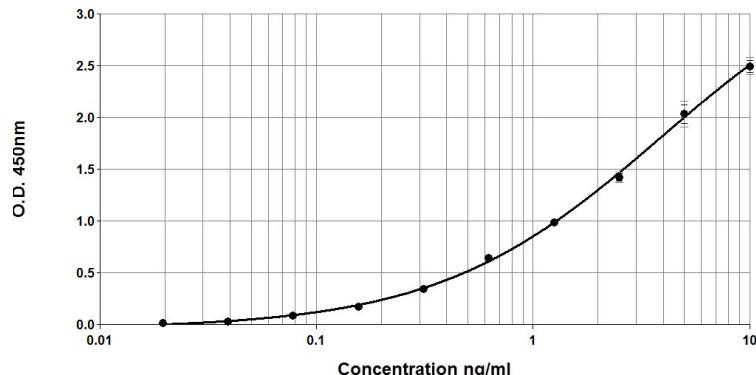
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Fel d 4 ELISA 2.0 Pre-coated Plate Kit

Product Code: EPC-FD4-X
Lot Number: xxxx

Sample curve:



Contents:

Microtiter plate coated with anti-Fel d 1 monoclonal antibody 5F3
Fel d 4 allergen standard (white cap)
Rabbit anti-Fel d 4 polyclonal antiserum (brown cap)
Peroxidase-conjugated goat anti-rabbit IgG (blue cap)
Wash buffer (10x concentrate)
Assay buffer (10x concentrate)
TMB developing substrate
Stop solution (0.5N sulfuric acid)

Store kit at 2-8°C

Expiry:

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

An InBio® product. Made in the USA.

Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody 5F3 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
Monoclonal Antibody: Immunogen: Isotype: Specificity:	5F3 Fel d 4 Mouse IgG1 Produced <i>in vitro</i> and purified by affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration:	1.19mg/ml in phosphate buffered saline, pH 7.4. Based on A280 for IgG (1.42=1mg/ml) 0.22 μ m filtered, preservative free.
Lot Number:	xxxxx
Detection Antibody: Immunogen: Isotype: Specificity:	Rabbit polyclonal antiserum Fel d 4 Multiple The antiserum contains IgG antibodies to cat <i>Felis domesticus</i> allergen, as well as IgG antibodies to other cat allergens.
Activity:	Titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22 μ m filtered, preservative free.
Lot Number:	xxxxx
Allergen Standard:	Recombinant Fel d 4 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	100ng/mL (based on amino acid analysis)
Lot Number:	xxxxx
Peroxidase-conjugated goat anti-rabbit:	
Lot Number:	xxxxx

Materials required, but not provided:

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

Data Processing:

4-parameter logistic curve fit (x-axis plotted on log scale)

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare a 1x dilution of wash and assay buffers from the 10x concentrates in clean containers using 18.2M Ω de-ionized water or Type I ultrapure water.
For one plate:
Wash buffer: add 15mL concentrate to 135mL water (150mL total volume)
Assay buffer: add 3mL concentrate to 27mL water (30mL total volume)
Adjust volumes accordingly for multi-plate assays. Diluted buffers may be stored at 4°C for up to 1 week.
2. Remove the plate from the foil pouch. Add 150 μ L wash buffer to each well. Empty the wells by inverting the plate and then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two times.
*Move directly to the next step to prevent the wells from drying.
3. Add 100 μ L assay buffer to all wells. Add an additional 80 μ L of assay buffer to wells A1-H1 (the total volume of assay buffer in these wells will be 180 μ L; all other wells will have 100 μ L).
4. **Standard:** gently vortex the standard and add 20 μ L to wells A1 and B1. Mix by pipetting up and down 8-10 times, and then transfer 100 μ L into wells A2 and B2. Mix and continue the doubling dilution scheme across the plate to wells A10 and B10. Remove and discard 100 μ L from wells A10 and B10 (100 μ L will remain).
The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks**.
Samples: add 20 μ L of sample to wells C1-H1. Mix by pipetting up and down 8-10 times. Transfer 100 μ L to wells C2-H2. Continue mixing and transferring to column 12. Remove and discard 100 μ L from wells C12-H12 (100 μ L will remain). When finished preparing the plate, the final volume in all wells should be 100 μ L.
5. Cover the plate and incubate for 1 hour \pm 10 minutes at room temperature (20-25°C) away from direct sunlight. **Note:** gentle agitation on a plate shaker during incubations may reduce variability.
6. Gently vortex the polyclonal detection antibody and peroxidase-conjugated goat anti-rabbit antibody. Prepare a 1:1,000 dilution mix by adding 11 μ L detection antibody and 11 μ L goat anti-rabbit antibody together in 11mL assay buffer in a reagent reservoir. **Mix thoroughly.** Wash the plate 3x with 150 μ L wash buffer per well. Add 100 μ L of the detection antibody/goat anti-rabbit antibody mix to each well.
7. Incubate the plate for 1 hour \pm 10 minutes at room temperature (20-25°C) away from direct sunlight.
8. Pour the TMB substrate and stop solution into separate reagent reservoirs so they are ready to use in Step 9. Wash the plate 3x with 150 μ L wash buffer per well.
9. Use a **multi-channel** pipette to add 100 μ L TMB to each well and monitor the reaction as the blue color develops. Once OD450 reaches 0.08-0.09 for Standard 1, use a **multi-channel** pipette to add 50 μ L stop solution to each well (the color will change to yellow).
10. Gently tap the plate to ensure homogeneity and measure the absorbance at 450nm within 30 minutes. The OD for Standard 1 should be between 1.2 and 3.5.