

Assay Performance Characteristics:

Standard range: 100-0.02ng/mL
Limit of Detection: 0.02ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

References:

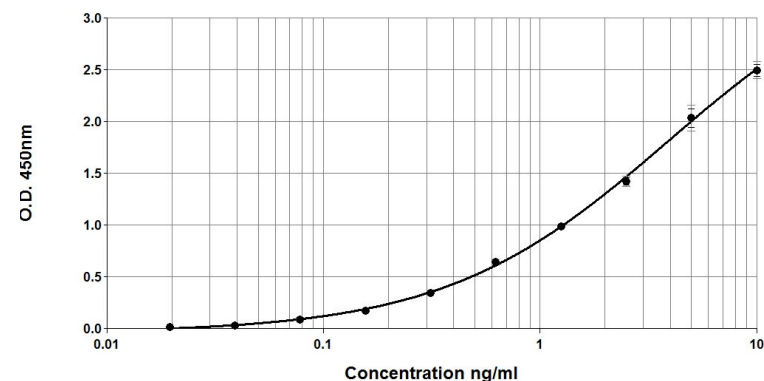
1. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997; 100(6 Pt 1):S2-24.
2. Platts-Mills TA, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001; 357(9258):752-756.



Fel d 4 ELISA 2.0 Pre-coated Plate Kit

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

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.

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

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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: 5F3
Immunogen: Fel d 4
Isotype: Mouse IgG1
Specificity: Produced *in vitro* 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) 0.22µm filtered, preservative free.

Lot Number: xxxxx

Detection Antibody: Rabbit polyclonal antiserum
Immunogen: Fel d 4
Isotype: Multiple
Specificity: The antiserum contains IgG antibodies to cat *Felis domesticus* 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, 0.22µm 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 18.2MΩ 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)

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. 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°C for up to 1 week
2. 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 then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two more times.
3. Add standards, samples, and blanks to the plate (final volume in all wells is 100µL).

Standards: add 180µL assay buffer into wells A1 and B1, and 100µL into remaining wells of rows A and B. Vortex the Fel d 4 standard and add 20µL to wells A1 and B1. Mix well by pipetting up and down 7-10 times and then transfer 100µL into wells A2 and B2. Mix well and continue the serial doubling dilution scheme across the plate to column 10.

The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks**.

Samples: Dust extracts are routinely tested starting at 1/10 dilution and may be prepared on the pre-coated plate: add 20µL sample to 180µL assay buffer in column A, mix, then transfer 100µL into 100µL assay buffer in the next well. Continue across the plate for the desired number of dilutions. A minimum of three dilutions per sample should be tested; 6-12 are recommended. Air filter extracts, allergen extracts, and other types of samples may require a different dilution scheme.

*Sample dilutions may also be prepared in tubes or on a 96-well dilution plate and transferred to the pre-coated plate.

4. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.
5. Wash the plate 3x with 150µL wash buffer per well. Vortex the polyclonal antibody and prepare a 1:1,000 detection antibody/conjugate mix by adding 11µL polyclonal antibody and 11µL peroxidase-conjugated goat anti-rabbit IgG to 11mL assay buffer.
Mix thoroughly and add 100µL to each well.
6. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.
7. Pour the TMB substrate and stop solutions into separate basins so they are ready to use in the next step. Wash the plate 3x with 150µL wash buffer per well.
8. Use a **multi-channel** pipette to add 100µL 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 50µL stop solution to each well (the color will change to yellow).
9. Read the plate at 450nm. The OD for Standard 1 should be between 1.2 and 3.5, with an ideal range of 2.0 - 2.5.