

## References:

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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.
3. Heinrich J, Bedada GB, Zock JP, Chinn S, Norback D, Oliveri M et al. Cat allergen level: its determinants and relationships to specific IgE to cat across European centers. J Allergy Clin Immunol 2006; 118(3):674-681
4. Jarvis D, Zock JP, Heinrich J, Svanes C, Verlato G, Oliveri M et al. Cat and dust mite allergen levels, specific IgG and IgG(4), and respiratory symptoms in adults. J Allergy Clin Immunol. 2007 Mar; 119(3):697-704.

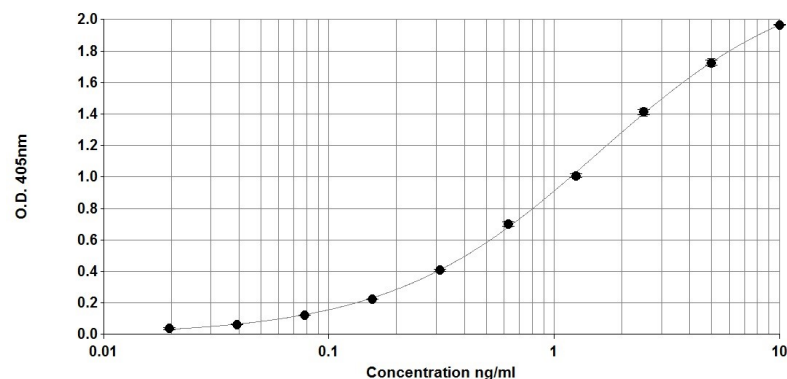


## Fel d 4 ELISA kit (5F3/FD4)

Product Code: EL-FD4

Lot Number: xxxxx

### Sample Curve:



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### Content:

- Vial 1 (red top) 100  $\mu$ L  
Monoclonal antibody 5F3  
Concentration: 1.19mg/ml in PBS
- Vial 2 (white top) 400  $\mu$ L  
rFel d 4 Standard  
Concentration: 100ng/ml Fel d 4
- Vial 3 (brown) 100  $\mu$ L  
Polyclonal rabbit antibody Fel d 4  
Dilute: 1:1000 for use

**Storage: The ELISA kit should be stored at 4°C**

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

**An InBio™ product**

## Certificate of Analysis

Monoclonal Antibody: 5F3  
Immunogen: Fel d 4  
Isotype: Mouse IgG1  
Specificity: Binds to an epitope on cat *Felis domesticus* allergen, Fel d 4.  
Purification: 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**

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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**

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Allergen Standard: rFel d 4  
Composition: Recombinant Fel d 4 prepared in 1% BSA/50% glycerol PBS, pH 7.4.  
Concentration: 100ng/ml rFel d 4  
**Lot Number: xxxxx**

## ELISA Protocol for Fel d 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 5F3 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.
2. 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.
3. Use doubling dilutions of the rFel d 4 Standard to make a control curve ranging from 10 - 0.02ng/ml Fel d 4: Pipette 20µl ST-FD4 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.
4. Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Fel d 4 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.
5. Wash wells 3x with PBS-T and add 100µl diluted polyclonal antiserum Fel d 4. The antibody solution contains 50% glycerol and should be diluted 1/1000 in 1%BSA, PBS-T. Incubate for 1 hour at room temperature.
6. Wash wells 3x with PBS-T and add 100µl diluted Peroxidase conjugated Goat anti-Rabbit IgG (Jackson Laboratories Cat#111-036-046, reconstituted in 1ml distilled water and 1ml glycerol). The reconstituted Goat anti-Rabbit IgG should be diluted 1/1000 in 1% BSA, PBS-T. Incubate for 1 hour at room temperature.
7. 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.**