

## References:

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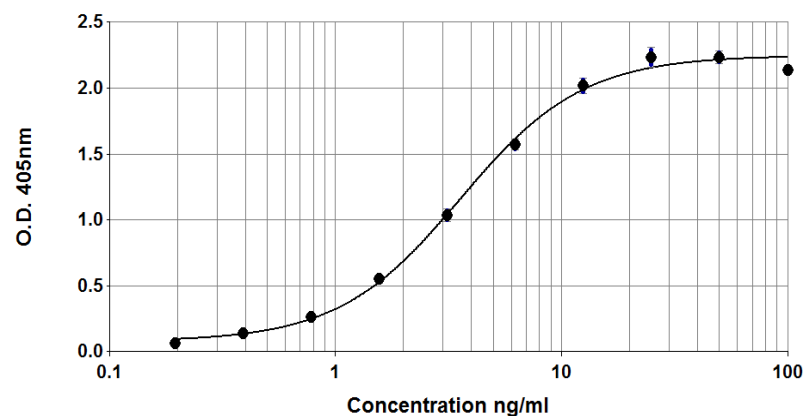


## Fel d 1 ELISA kit (6F9/3E4)

Product Code: EL-FD1

Lot Number: xxxxx

### Sample Curve:



### Content:

Vial 1 (red top) 100  $\mu$ L  
Monoclonal antibody 6F9  
Concentration: 1mg/ml in PBS

Vial 2 (white top) 400  $\mu$ L  
Universal Allergen Standard  
Concentration: 1000ng/ml Fel d 1

Vial 3 (brown) 100  $\mu$ L  
Biotinylated Monoclonal antibody 3E4  
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.**

## Certificate of Analysis

**Monoclonal Antibody:** 6F9 (clone 6F9 A4 H1)  
**Immunogen:** Fel d 1  
**Isotype:** Mouse IgG1  
**Specificity:** Binds to an epitope on cat *Felis domesticus* allergen, Fel d 1, Chain 1.  
**Purification:** Produced in tissue culture and purified by affinity chromatography using recombinant Protein G. Single heavy and light chain bands on SDS-PAGE.  
**Concentration:** 1.0mg/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:** 3E4 (clone 3E4 C4 C10)  
**Immunogen:** Fel d 1  
**Isotype:** Mouse IgG1  
**Specificity:** Binds to an epitope on cat *Felis domesticus* allergen, Fel d 1, Chain 1.  
**Purification:** Produced in ascites and purified by ammonium sulphate precipitation and HPLC using recombinant 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
Der f 1	Amino-acid analysis	2500
Der p 2	Amino-acid analysis	1000
<b>Fel d 1</b>	<b>Amino-acid analysis</b>	<b>1000</b>
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 Fel d 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 6F9 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 Universal Allergen Standard to make a control curve ranging from 100 - 0.2ng/ml Fel d 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.
4. Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Fel d 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.
5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Fel d 1 mAb 3E4. 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 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.
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.**