

References

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www.inbio.com

Indoor Biotechnologies, Inc.
1216 Harris Street,
Charlottesville
Virginia, 22903
United States

Tel: (434) 984-2304
Fax: (434) 984-2709
mail@inbio.com

Indoor Biotechnologies Ltd
The Old Brewery
38, High Street, Warminster
Wiltshire, BA12 9AF
United Kingdom

Tel: 44 (0)5601 153 291
Fax: 44 (0)1985 218 300
info@indoorbiotech.co.uk

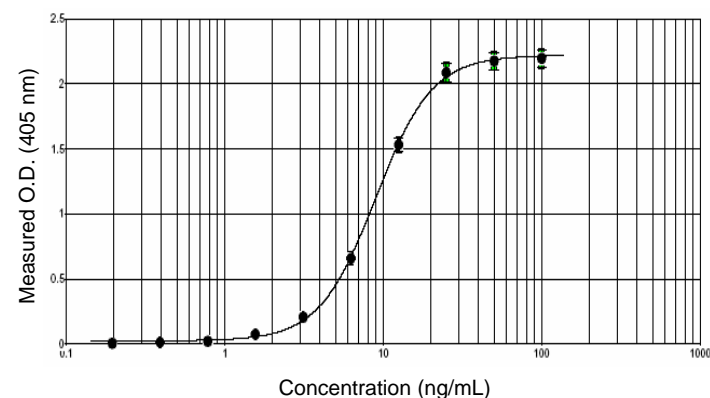


Fel d 1 ELISA kit (6F9/3E4)

Product Code: EL-FD1

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 200 μ L
Monoclonal antibody 6F9
Concentration: 1mg/ml in PBS

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

Vial 3 (brown) 200 μ L
Biotinylated monoclonal antibody 3E4
Dilute: 1:1000 for use

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

For Research Use Only: Not for Diagnostic 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 present on Fel d 1 Chain 1.
Purification: Produced in tissue culture and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration: 1mg/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 present on Fel d 1 Chain 1.
Purification: From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. Single heavy and light chain bands on SDS-PAGE
Biotinylation: Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent and titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4
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
Concentration/Calibration:
Lot Number xxxxx

| 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 |
| Fel d 1 | Amino-acid analysis | 1000 |
| 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.

- Anti-Fel d 1 mAb 6F9 is supplied HPLC purified as a stock solution in PBS (Product Code: MA-6F9). Dilute the mAb 6F9 1/1000 (i.e. 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog #439454, Fisher Catalog #12565135) with 100µl of diluted mAb 6F9 per well. Incubate overnight at 4°C.
- Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30min at room temperature with 100µl 1% BSA-PBS-T. Wash 3x with PBS-T.
- Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - Make a Fel d 1 control curve using doubling dilutions of the Universal Allergen Standard (Product Code: ST-UAS): The control curve dilutions are from 100-0.2 ng/ml Fel d 1. Pipette 20µl allergen standard into 180µl 1% BSA PBS-T into wells A1 and B1 of 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 and B12 should contain only 1% BSA PBS-T as blanks.
 - House dust samples are routinely diluted two-fold from 1/10 -1/80.
- Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Fel d 1 mAb 3E4 (Product Code: BI-3E4). The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA-PBS-T. Incubate for 1 hour at room temperature.
- 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 (i.e. 10µl/10ml) in 1% BSA PBS-T. Incubate for 30 minutes at room temperature.
- Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H₂O₂ (i.e. 10µl/10ml ABTS). Read the plate when the optical density 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.

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