

References

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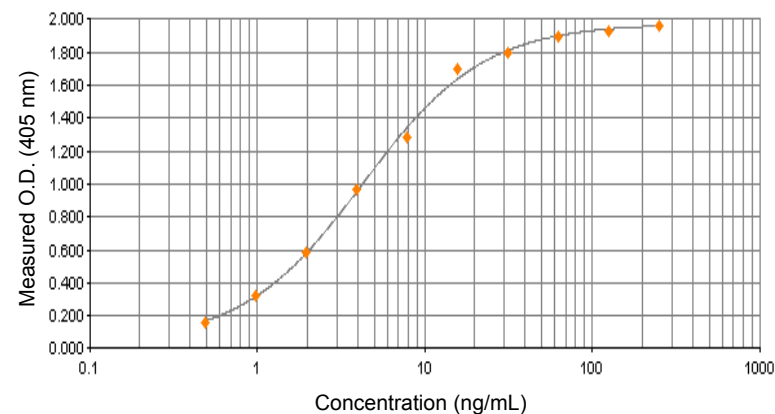
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Can f 1 ELISA kit

Product Code: EL-CF1
Lot Number: 34010

Sample Curve:



Content:

- Vial 1 (red top) 200 μ L
Monoclonal antibody 10D4
Concentration: 1mg/ml in PBS
- Vial 2 (white top) 2 x 400 μ L
Universal Allergen Standard
Concentration: 2500ng/ml Can f 1
- Vial 3 (brown) 200 μ L
Rabbit anti. Can f 1
Dilute: 1:1000 for use

Storage: Freeze ELISA standard on arrival and store the remainder of kit at 4°C

For Research Use Only: Not for Diagnostic or Therapeutic Use

Certificate of Analysis

Monoclonal Antibody: 10D4
 Immunogen: Can f 1
 Isotype: Mouse IgG2ak
 Specificity: Binds to an epitope present on Can f 1.
 Purification: Produced in tissue culture and purified by affinity 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: 30064

Antibody: Polyclonal rabbit antiserum raised against dog extract
 Specificity: The antiserum contains IgG antibodies to Can f 1 as well as IgG antibodies to other dog allergens
 Activity: The antiserum has been diluted in phosphate buffered saline containing 1%BSA 50%glycerol PBS, pH 7.4. The antiserum has been filtered and should be diluted 1/1000 for use in Can f 1 assays.

Lot Number: 2892

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 31109

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 Can f 1.

- Anti-Can f 1 mAb 10D4 is supplied HPLC purified as a stock solution in PBS (Product Code: MA-10D4). Dilute the mAb 10D4 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 the diluted mAb 10D4 per well. Incubate overnight at 4°C.
- Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100 µl 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 Can f 1 control curve using doubling dilutions of the Universal Allergen Standard (Product Code: ST-UAS): The control curve dilutions are from 250-0.5ng/ml Can f 1. Pipette 20µl 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, 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 Rabbit anti-Can f 1 antibody (Product Code: PA-CF1). 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 Peroxidase conjugated Goat anti-Rabbit IgG (Jackson Laboratories Cat. #111-036-046, reconstituted in 1ml distilled water and 1 ml glycerol). The reconstituted Goat anti Rabbit 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 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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