References:

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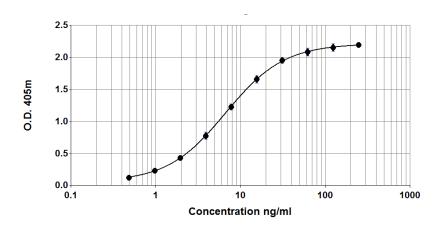
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Bla g 2 ELISA kit (7C11/BG2)

Product Code: EL-BG2 Lot Number: XXXXX

Sample Curve:



Content:

(red top) 100 µL Monoclonal antibody 7C11

Vial 2 (white top) 400 ul Universal Allergen Standard Concentration: 2500ng/ml

(brown) 100 uL Vial 3 Polyclonal Rabbit anti Bla q 2 antibody 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: 7C11 (clone 7C11 C2 A6)

Immunogen: Bla g 2 Isotype: Mouse IgG1

Specificity: Binds to an epitope on cockroach Blattella germanica

allergen, Bla g 2.

Purification: Produced in ascites and purified by ammonium sulphate

precipitation and affinity chromatography using Protein A.

Single heavy and light chain bands on SDS-PAGE.

Concentration: 1 mg/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

Antibody: Rabbit polyclonal antiserum

Immunogen: Recombinant Bla g 2

Isotype: Multiple

Specificity: The antiserum contains IgG antibodies to cockroach

Blattella germanica allergen, Bla g 2 and does not

react with Bla q 1.

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: xxxx

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
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 Bla g 2.

- 1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 7C11 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, 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 100μl/well of 1% BSA, PBS-T. Wash 3x with PBS-T.
- 3. Use doubling dilutions of the Universal Allergan standard to make a control curve ranging from 250 0.5ng/ml Bla g 2: 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 Bla g 2 analysis are routinely diluted twofold from1/10-1/80. Other sample types, like air filter extracts and allergen extracts, may require different dilutions.
- Wash wells 3x with PBS-T and add 100µl diluted polyclonal Rabbit anti Bla g 2 antibody. 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 1 ml distilled water and 1ml glycerol). The reconstituted Goat anti-Rabbit IgG should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA, PBS-T. Incubate for 1hour at room temperature.
- 7. Wash wells 3x and develop the assays by adding $100\mu I$ 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H_2O_2 . Read the plate when the absorbance at 405nm reaches 2.0-2.4.

Notes:

The Bla g 2 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.

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