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Blag 2 ELISA kit (7C11/BG2)

Product Code: EL-BG2 Lot Number: XXXXX

Sample Curve:



Content:

- Vial 1 (red top) 100 µL Monoclonal antibody 7C11
- Vial 2 (white top) 400 µl Universal Allergen Standard Concentration: 2500ng/ml
- Vial 3 (brown) 100 µL Polyclonal Rabbit anti Bla g 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

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Certificate of Analysis

7C11 (clone 7C11 C2 A6) Bla g 2 Mouse IgG1 Binds to an epitope on cockroach <i>Blattella germanica</i> allergen, Bla g 2. Produced in ascites and purified by ammonium sulphate precipitation and affinity chromatography using Protein A.
Single heavy and light chain bands on SDS-PAGE. 1 mg/ml in phosphate buffered saline, pH 7.4. Based on A280 for IgG (1.42=1mg/ml), 0.22µm filtered, preservative free.
xxxxx
Rabbit polyclonal antiserum Recombinant Bla g 2 Multiple The antiserum contains IgG antibodies to cockroach <i>Blattella germanica</i> allergen, Bla g 2 and does not
react with Bla g 1. Titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
XXXX
Universal Allergen Standard A formulation of eight purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

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.

- 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.
- 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.
- 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.
- 5. 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 μ l 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H₂O₂. 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.