References:


Bla g 2 ELISA kit (7C11/BG2)

Product Code: EL-BG2
Lot Number: xxxxxx

Sample Curve:

Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 7C11
Concentration: 1mg/ml in PBS

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.
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 g 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: 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

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.
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 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 two-fold 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 100μl 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.

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