

References:

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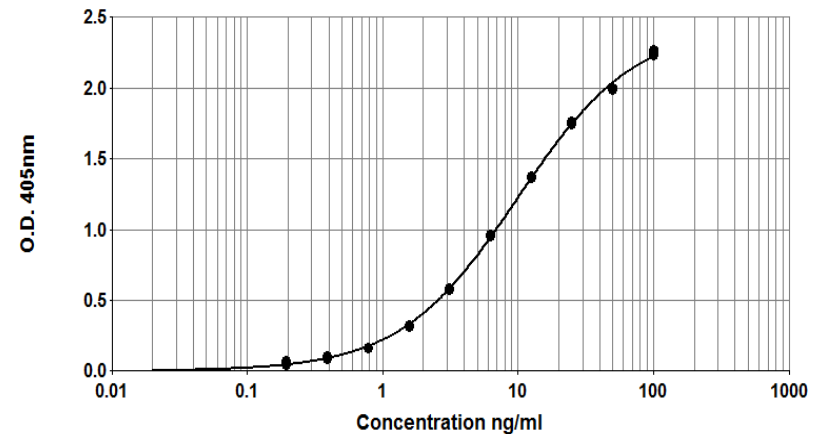


Bla g 1 ELISA kit (10A6/BG1)

Product Code: EL-BG1

Lot Number: xxxxx

Sample Curve:



Content:

- Vial 1 (red top) 100 μ L
Monoclonal antibody 10A6
Concentration: 1mg/ml in PBS
- Vial 2 (white top) 400 μ L
Bla g 1 Standard
Concentration: 1000ng/ml Bla g 1
- Vial 3 (brown) 100 μ L
Rabbit anti Bla g 1 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: 10A6 (clone 10A6 F5 E1)
Immunogen: Bla g 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on cockroach *Blattella germanica* allergen Bla g 1 and *Periplaneta americana* allergen, Per a 1.
Purification: From ascites by ammonium sulphate precipitation and by HPLC using recombinant protein G. 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: Polyclonal antibody raised against recombinant Bla g 1
Specificity: The pAb contains IgG antibodies to Bla g 1 and does not react with Bla g 2.
Activity: The pAb is in phosphate buffered saline, pH 7.4, containing 1%BSA/50% glycerol. The pAb has been 0.22µm filtered and should be diluted 1/1000 for use in Bla g 1 ELISA.
Lot Number: **xxxx**

Allergen Standard: rBla g 1
Composition: Purified from *Pichia pastoris* culture by affinity chromatography and prepared in 1% BSA, 50% glycerol/PBS, pH 7.4.
Concentration: 1000ng/ml
Calibration: The concentration of the purified recombinant Bla g 1 was determined by amino acid analysis. There are no national or international reference standards for cockroach allergens.
Lot Number: **xxxxx**

Note: *Bla g 1 measurements were previously reported in arbitrary units (U/ml). Studies carried out with rBla g 1, Lot 36019 have determined that 1 Unit is ~100ng Bla g 1 protein.*

ELISA Protocol for Bla g 1.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 10A6 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 rBla g 1 standard to make a control curve ranging from 100 - 0.2ng/ml Bla g 1: Pipette 20µl Bla g 1 standard 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 1 analysis are routinely diluted two-fold from 1/10-1/80. 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 1 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 1 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.