

References

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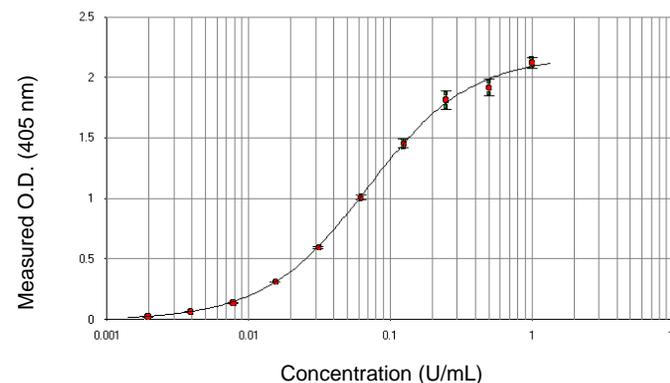


Bla g 1 ELISA kit

Product Code: EL-BG1

Lot Number: xxxxx

Sample Curve:



4 Parameters $y = (a-d)/(1+(x/c)^b) + d$
a=0.006065 b=1.193 c=0.06951 d=2.174
R=0.9995 R²=0.9989 err=0.02548

Content:

- Vial 1 (red top) 200 μ L
Monoclonal antibody 10A6
Concentration: 1mg/ml in PBS
- Vial 2 (white top) 800 μ L
Bla g 1 Standard
Concentration: 10U/ml Bla g 1
- Vial 3 (brown) 200 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 Use Only: Not for Diagnostic or Therapeutic Use

Certificate of Analysis

Monoclonal Antibody: 10A6 (clone 10A6 F5 E1)
Product Code: MA-10A6
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: **xxxx**

Antibody: Polyclonal rabbit antiserum raised against recombinant Bla g 1
Product Code: PA-BG1
Specificity: The antiserum contains IgG antibodies to Bla g 1 and does not react with Bla g 2.
Activity: The antiserum has been diluted in phosphate buffered saline, pH 7.4, containing 1%BSA/50% glycerol. The antiserum has been filtered and should be diluted 1/1000 for Bla g 1 ELISA.
Lot Number: **xxxx**

Allergen Standard: Cockroach (*B. gemanica*)
Product Code: ST-BG1
Composition: *Blattella gemanica* fras extract was prepared in 1% BSA, 0.01% thimerosal, 0.05% tween/ 50% glycerol/phosphate buffered saline, pH7.4.
Concentration: 10 Units /ml Bla g 1
Calibration: The extract was sub-standardized by ELISA against a *B. germanica* standard prepared in Dr. Chapman's laboratory at the Asthma and Allergic Disease Center, University of Virginia. The extract is calibrated in arbitrary units. There are no national or international reference standards for cockroach allergens.
Lot Number: **xxxxx**

ELISA Protocol for Bla g 1.

1. Anti-Bla g 1 mAb 10A6 is supplied HPLC purified as a stock solution at 1mg/ml in PBS (Product Code: MA-10A6). Dilute the mAb 10A6 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 diluted mAb 10A6 per well. Incubate overnight at 4°C.
2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30min at room temperature with 100µl 1% BSA-PBS-T. Wash 3x with PBS-T.
3. Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - 3a. Make a Bla g 1 control curve using doubling dilutions of the Bla g 1 Standard (Product Code: ST-BG1): The control curve dilutions are from 1-0.002U/ml Bla g 1. Pipette 20µl allergen 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, and A12 and B12 should contain only 1% BSA PBS-T as blanks.
 - 3b. House dust samples are routinely diluted two-fold from 1/10 -1/80.
4. Wash wells 3x with PBS-T and add 100µl diluted Rabbit anti. Bla g 1 antibody (Product Code: PA-BG1). 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.
5. 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 should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA PBS-T. Incubate for 1hour at room temperature.
6. 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:

Bla g 1 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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